(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 October 2002 (10.10.2002)

PCT

(10) International Publication Number
WO 02/079238 A2

(51) International Patent Classification[7]: C07K 14/00

(21) International Application Number: PCT/EP02/03540

(22) International Filing Date: 28 March 2002 (28.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

01108315.1	2 April 2001 (02.04.2001)	EP
01113419.4	1 June 2001 (01.06.2001)	EP

(71) Applicant *(for all designated States except US)*: **DEVELOGEN AKTIENGESELLSCHAFT FÜR ENTWICKLUNGSBIOLOGISCHE FORSCHUNG** [DE/DE]; Rudolf-Wissell-Strasse 28, 37079 Göttingen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **EULENBERG, Karsten** [DE/DE]; Am Graben 10b, 37079 Göttingen (DE). **BRÖNNER, Günter** [DE/DE]; Springstrasse 54, 37077 Göttingen (DE). **CIOSSEK, Thomas** [DE/DE]; Kiesseestrasse 49a, 37083 Göttingen (DE). **HÄDER, Thomas** [DE/DE]; Wiesenstr. 17, 37073 Göttingen (DE). **STEUERNAGEL, Arnd** [DE/DE]; Am Kirschberge 4, 37085 Göttingen (DE).

(74) Agents: **WEICKMANN, Franz, Albert** et al.; Weickmann & Weickmann, Postfach 860 820, 81635 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/079238 A2

(54) Title: PROTEIN DISULFIDE ISOMERASE AND ABC TRANSPORTER HOMOLOGOUS PROTEINS INVOLVED IN THE REGULATION OF ENERGY HOMEOSTASIS

(57) Abstract: The present invention discloses three novel proteins regulating the energy homeostasis and the metabolism of triglycerides, and polynucleotides, which identify and encode the proteins disclosed in this invention. The invention also relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides of the invention. The invention also relates to the use of these sequences in the diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation, for example, but not limited to, metabolic diseases such as obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, pancreatic dysfunctions, arteriosclerosis, coronary artery disease (CAD), coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and others.

Protein disulfide isomerase and ABC transporter homologous proteins involved in the regulation of energy homeostasis

Description

This invention relates to the use of nucleic acid and amino acid sequences of protein disulfide isomerase and ABC transporter homologous proteins, and to the use of these sequences in the diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation, for example, but not limited to, metabolic diseases such as obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and others.

Obesity is one of the most prevalent metabolic disorders in the world. It is still poorly understood human disease that becomes more and more relevant for western society. Obesity is defined as an excess of body fat, frequently resulting in a significant impairment of health. Besides severe risks of illness such as diabetes, hypertension and heart disease, individuals suffering from obesity are often isolated socially. Human obesity is strongly influenced by environmental and genetic factors, whereby the environmental influence is often a hurdle for the identification of (human) obesity genes. Obesity is influenced by genetic, metabolic, biochemical, psychological, and behavioral factors. As such, it is a complex disorder that must be addressed on several fronts to achieve lasting positive clinical outcome. Obese individuals are prone to ailments including: diabetes mellitus, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, and sleep apnea.

Obesity is not to be considered as a single disorder but a heterogeneous group of conditions with (potential) multiple causes. Obesity is also characterized by elevated fasting plasma insulin and an exaggerated insulin response to oral glucose intake (Koltermann, J. Clin. Invest 65, 1980, 1272-1284) and a clear involvement of obesity in type 2 diabetes mellitus can be confirmed (Kopelman, Nature 404, 2000, 635-643).

Even if several candidate genes have been described which are supposed to influence the homeostatic system(s) that regulate body mass/weight, like leptin, VCPI, VCPL, or the peroxisome proliferator-activated receptor-gamma co-activator, the distinct molecular mechanisms and/or molecules influencing obesity or body weight/body mass regulations are not known.

Therefore, the technical problem underlying the present invention was to provide for means and methods for modulating (pathological) metabolic conditions influencing body-weight regulation and/or energy homeostatic circuits. The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to genes with novel functions in body-weight regulation, energy homeostasis, metabolism, and obesity. The present invention discloses specific genes involved in the regulation of body-weight, energy homeostasis, metabolism, and obesity, and thus in disorders related thereto such as eating disorder, cachexia, diabetes mellitus, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, and sleep apnea. The present invention describes human protein disulfide isomerase, MRP4, and ABC8 (white) genes as being involved in those conditions mentioned above.

Protein disulfide isomerase (PDI) is a protein-thiol oxidoreductase that catalyzes the folding of protein disulfides. PDI has been demonstrated to

participate in the regulation of reception function, cell-cell interaction, gene expression, and actin filament polymerization. PDI has acts as a chaperone and subunit of prolyl 4-hydroxylase and microsomal triglyceride transfer protein (MTP). MTP is accelerating the transport of triglyceride, cholesteryl ester, and phospholipid between membranes (Berriot-Varoqueaux et al., 2000, *Annu Rev Nutr.* 20, 663-697). Mutations in MTP are the cause for abetalipoproteinemia, a hereditary disease with limited production of chylomicrons and very low-density lipoproteins (VLDL) in the intestine and liver (Rehberg et al., 1996, *J Biol Chem.* 271(47), 29945-52). Intracellular VLDL is associated with chaperones including PDI and glucose regulated protein 94 (GRP94, endoplasmin) and assembles with apolipoprotein B (Berriot-Varoqueaux et al., SUPRA). These chaperones are endogenous substrates of sphingosine-dependent kinases (SDKs) and regulated by signal transduction pathways (see, for example, Megidish et al., 1999, *Biochemistry.* 38(11), 3369-78).

The chaperones are found in the endoplasmic reticulum where the lipidation of lipoproteins like apolipoprotein B might take place (Hussain et al., 1997, *Biochemistry.* 36(42), 13060-7.; Wu et al., 1996, *J Biol Chem.* 271(17), 10227-81). In addition, the secretion of apolipoprotein B is dependent on PDI (Wang et al., 1997, *J. Biol. Chem.* 272(44), 27644-51).

The formation or assembly of additional proteins strongly depends on the activity of specific groups of chaperones. PDI is regulating the formation of native insulin from its precursors and the insulin degradation (Tang et al., 1988, *Biochem J.* 255(2), 451-5; Duckworth et al., 1998, *Endocr Rev.* 19(5), 608-24). Insulin signaling is crucial for the proper regulation of blood glucose levels and lipid metabolism.

Dietary energy tissue-specifically regulates endoplasmic reticulum chaperone gene expression in the liver of mice, especially glucose regulated proteins (Dhahbi et al., 1997, *J Nutr.* 127(9), 1758-64).

Additionally PDI mRNA is strongly expressed in adipose tissue (Klaus et al., 1990, *Mol Cell Endocrinol.* 73(2-3), 105-10), emphasizing their important roles in metabolic pathways.

Chaperones are also essential for the cellular protection against stress in its different forms like oxidative, heat shock or hypoglycemic stress (Barnes & Smoak, 2000, *Anat Embryol.* 202(1), 67-74, Lee, 1992. *Curr Opin Biol.* 4(2), 267-73) preventing cells to undergo apoptosis.

Furthermore, chaperones are involved in different diseases like Alzheimer's Disease (Yoo et al., 2001, *Biochem Biophys Res Commun.* 280(1), 249-58), Parkinson (Duan & Mattson, 1999, *J Neurosci Res.* 59(13), 195-206), Rheumatoid Arthritis (Corrigall et al., 2001, *J Immunol.* 166(3), 1492-98) or neuropsychological disease leading to suicide of patients (Bown et al., 2000, *Neuropsychopharmacology.* 22(3), 327-32).

ATP-binding cassette (ABC) genes encode a family of transport proteins that are known to be involved in a number of human genetic diseases. For example, polymorphisms of the human homologue of the *Drosophila white* gene are associated with mood and panic disorders (Nakamura et al. 1999 *Mol Psychiatry.* 4(2):155-62). Mutations in the canilicular multispecific organic anion transporter (cMOAT) gene could be the reason for the Dubin-Johnson syndrome leading to hyperbilirubinemia II (Wada et al. 1998, *Hum Mol Genet.* 7(2):203-7). The rod photoreceptor-specific ABC transporter (ABCR) is responsible for the Stargardt disease (Allikmets et al. 1997, *Nat Genet.* 15(3):236-46). The gene encoding ATP-binding cassette transporter 1 (ABC1) is mutated in Tangier disease leading to the absence of plasma high-density lipoprotein (HDL) and deposition of cholesteryl esters in the reticulo-endothelial system with splenomegaly and enlargement of tonsils and lymph nodes (Brooks-Wilson et al. 1999 *Nat Genet.* 22(4):336-45, Bodzioch et al. 1999 *Nat Genet.* 22(4):347-51, Rust et al. 1999, *Nat Genet.* 22(4):352-5). Furthermore a subgroup of ABC

transporters are involved in cellular detoxification causing multidrug resistance that counteracts e.g. anti-cancer or HIV treatment (Schuetz et al. 1999 *Nat Med.* 1999 (9):1048-51).

ABC transporters transport several classes of molecules. ABC1 (ABCA1), the gene mutated in Tangier disease, mediates apoAI associated export of cholesterol and phospholipids from the cell and is regulated by cholesterol efflux ((Brooks-Wilson et al. 1999 *Nat Genet.* 22(4):336-45, Bodzioch et al. 1999 *Nat Genet.* 22(4):347-51, Rust et al. 1999, *Nat Genet.* 22(4):352-5Brooks-Wilson et al. 1999, Bodzioch et al. 1999, Rust et al. 1999). ABC1 is expressed on the plasma membrane and Golgi complex and the lipid export process needs vesicular budding between Golgi and plasma membrane that is disturbed in Tangier disease. LDL and HDL_3 regulate the expression of ABC1 in macrophages (Orsó et al. 2000 *Nat Genet.* 24:192-6).

The expression of the human homologue of the *Drosophila white* gene (ABC8 or ABCG1) is induced in monocyte-derived macrophages during cholesterol influx mediated by LDL and is downregulated through lipid efflux mediated by cholesterol acceptor HDL_3. ABC8 is expressed on the cell surface and intracellular compartments of cholesterol-loaded macrophages and its expression is also regulated by oxysterols that act through nuclear oxysterol receptors, liver X receptors (LXRs) and by retinoid X receptor ligand. Therefore, ABC8 activity in macrophages might be crucial for cholesterol metabolism and the development of arteriosclerosis similar to ABC1. LXR expression is regulated through PPARg gamma signalling that is essential for adipogenesis and therefore PPARg gamma signalling might regulate the expression of ABC1 and ABC8 transporters at least in macrophages (Klucken et al. 2000 *Proc Natl Acad Sci USA.* 97(2):817-22., Venkateswaran et al. 2000 *J Biol Chem.* 275(19):14700-7).

Another subgroup of ABC transporters mediates cellular detoxification and is therefore named Multidrug Resistance-associated Proteins (MRPs) or canilicular Multispecific Organic Anion Transporters (cMOATs). MRP1 has high activity towards compounds conjugated to glutathione (GSH), glucuronide or sulfate and transports sphingolipids, eicosanoids, phosphatidylcholine and phosphatidylethanolamine analogues but the main function is the cellular detoxification. MRP4 (MOAT-B) overexpression is associated with high-level resistance to the nucleoside analogues 9-(2-phosphonylmethoxyethyl)adenine and azidothymidine monophosphate, both of which are used as anti-human immunodeficiency virus (HIV) drugs (Schuetz et al. 1999 *Nat Med.* 5(9):1048-51). MRP4 function in lipid transport is unknown despite the fact that LDL and HDL_3 regulate its expression in macrophages.

So far, it has not been described that protein disulfide isomerase or ABC transporters and the homologous human proteins are involved in the regulation of energy homeostasis and body-weight regulation and related disorders, and thus, no functions in metabolic diseases and other diseases as listed above have been discussed. In this invention we demonstrate that the correct gene doses of protein disulphide isomerase and/or of two ABC transporter genes are essential for maintenance of energy homeostasis. A genetic screen was used to identify that the protein disulfide isomerase gene, the MRP4 gene, and/or the white (ABC8) gene cause obesity in *Drosophila melanogaster*, reflected by a significant increase of triglyceride content, the major energy storage substance.

Polynucleotides encoding proteins with homologies to protein disulfide isomerase or ABC transporters present the opportunity to investigate diseases and disorders, including metabolic diseases and disorders such as obesity, as well as related disorders such as described above. Molecules related to protein disulfide isomerase and ABC transporters satisfy a need in the art by providing new compositions useful in diagnosis, treatment,

and prognosis of diseases and disorders, including metabolic diseases and disorders such as described above.

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure.

The present invention discloses a novel protein disulfide isomerase homologous protein and two novel ABC transporter homologous proteins regulating the energy homeostasis and the fat metabolism, especially the metabolism and storage of triglycerides, and polynucleotides, of triglycerides, and polynucleotides, which identify and encode the proteins disclosed in this invention. The invention also relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides of the invention. The invention also relates to the use of these sequences in the diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation, for example, but

not limited to, metabolic diseases such as obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and others.

Protein disulfide isomerase and ABC transporter homologous proteins and nucleic acid molecules coding therefor are obtainable from insect or vertebrate species, e.g. mammals or birds. Particularly preferred are human protein disulfide isomerase and ABC transporter homologous nucleic acids, particularly nucleic acids encoding human protein disulfide isomerase homologous protein (MGC3178; Genbank Accession No. NM_030810 (identical to Genbank Accession No. BC001199) for the mRNA; NP_110437.1 (identical to Genbank Accession No. AAH01199) for the protein), human ATP-binding cassette, subfamily C (CFTR/MRP), member 4 (ABCC4; MRP4; Genbank Accession No. NM_005845 for the mRNA; NP_005836.1 for the protein), and human ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1; WHITE; Genbank Accession No. XM_009777 for the mRNA; XP_009777.3 for the protein). Also particularly preferred are *Drosophila* protein disulfide isomerase homologous and ABC transporter homologous nucleic acids and polypeptides encoded thereby. In a preferred embodiment the present invention also comprises so-called "ABC-tran" domains of the proteins and nucleic acid molecules coding therefor.

The invention particularly relates to a nucleic acid molecule encoding a polypeptide contributing to regulating the energy homeostasis and the metabolism of triglycerides, wherein said nucleic acid molecule comprises

(a) the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5,

(b) a nucleotide sequence which hybridizes at 66°C in a solution containing 0.2 x SSC and 0.1% SDS to the complementary strand of a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6,

(c) a sequence corresponding to the sequences of (a) or (b) within the degeneration of the genetic code,

(d) a sequence which encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at least 98% and up to 99,6% identical to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6,

(e) a sequence which differs from the nucleic acid molecule of (a) to (d) by mutation and wherein said mutation causes an alteration, deletion, duplication or premature stop in the encoded polypeptide or

(f) a partial sequence of any of the nucleotide sequences of (a) to (e) having a length of at least 15 bases, preferably at least 20 bases, more preferably at least 25 bases and most preferably at least 50 bases.

The invention is based on the discovery that protein disulfide isomerase homologous proteins (particularly PDI-like; referred to as DevG20) and ABC transporter homologous proteins (particularly MRP4 and WHITE; herein referred to as DevG4 and DevG22, respectively) and the polynucleotides encoding these, are involved in the regulation of triglyceride storage and therefore energy homeostasis. The invention describes the use of these compositions for the diagnosis, study, prevention, or treatment of diseases and disorders related to such cells, including metabolic diseases such as obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and others.

To find genes with novel functions in energy homeostasis, metabolism, and obesity, a functional genetic screen was performed with the model organism *Drosophila melanogaster* (Meigen). One resource for screening was a proprietary *Drosophila melanogaster* stock collection of EP-lines. The P-vector of this collection has Gal4-UAS-binding sites fused to a basal promoter that can transcribe adjacent genomic *Drosophila* sequences upon binding of Gal4 to UAS-sites. This enables the EP-line collection for overexpression of endogenous flanking gene sequences. In addition, without activation of the UAS-sites, integration of the EP-element into the gene is likely to cause a reduction of gene activity, and allows determining its function by evaluating the loss-of-function phenotype.

Triglycerides are the most efficient storage for energy in cells. In order to isolate genes with a function in energy homeostasis, several thousand proprietary EP-lines were tested for their triglyceride content after a prolonged feeding period (see Examples for more detail). Lines with significantly changed triglyceride content were selected as positive candidates for further analysis.

Obese people mainly show a significant increase in the content of triglycerides. In this invention, the content of triglycerides of a pool of flies with the same genotype after feeding for six days was analyzed using a triglyceride assay, as, for example, but not for limiting the scope of the invention, is described below in the EXAMPLES section.

The invention encompasses polynucleotides that encode *DevG20*, *DevG4*, *DevG22*, and homologous proteins. Accordingly, any nucleic acid sequence, which encodes the amino acid sequences of DevG20, DevG4, or DevG22, can be used to generate recombinant molecules that express DevG20, DevG4, or DevG22. In a particular embodiment, the invention encompasses the nucleic acid sequence of 1693 base pairs (PDI, referred to as *DevG20* SEQ ID NO:1) as shown in FIG. 4A, the nucleic acid

sequence of 4487 base pairs (MRP4, referred to as *DevG4,* SEQ ID NO:3) as shown in FIG. 9A, and the nucleic acid sequence of 2459 base pairs (ABC8, or white, referred to as *DevG22* SEQ ID NO:5) as shown in FIG. 15A. It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding *DevG20, DevG4,* or *DevG22,* some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequences of naturally occurring *DevG20, DevG4, or DevG22,* and all such variations are to be considered as being specifically disclosed. Although nucleotide sequences which encode *DevG20, DevG4, or DevG22* and variants thereof are preferably capable of hybridising to the nucleotide sequences of the naturally occurring *DevG20, DevG4, or DevG22* under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding *DevG20, DevG4, or DevG22* or derivatives thereof possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilised by the host. Other reasons for substantially altering the nucleotide sequence encoding *DevG20, DevG4, or DevG22* and derivatives thereof without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequences. The invention also encompasses production of DNA sequences, or portions thereof, which encode *DevG20, DevG4, or DevG22* and derivatives thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art at the time of the filing of this

application. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding *DevG20, DevG4, or DevG22* or any portion thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridising to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:1, SEQ ID NO:3, and in SEQ ID NO:5, under various conditions of stringency. Hybridisation conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe, as taught in Wahl, G. M. and S. L. Berger (1987: Methods Enzymol. 152:399-407) and Kimmel, A. R. (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency. Preferably, hybridization under stringent conditions means that after washing for 1 h with 1 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 68°C, particularly for 1 h in 0.2 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 68°C, a positive hybridization signal is observed. Altered nucleic acid sequences encoding *DevG20, DevG4, or DevG22* which are encompassed by the invention include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent protein.

The encoded proteins may also contain deletions, insertions, or substitutions of amino acid residues, which produce a silent change and result in a functionally equivalent protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of DevG20, DevG4, or DevG22 is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine,

and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

Also included within the scope of the present invention are alleles of the genes encoding *DevG20, DevG4, or DevG22*. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene, which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structures or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes, which give rise to alleles, are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence. Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE DNA Polymerase (US Biochemical Corp, Cleveland Ohio), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, Ill.), or combinations of recombinant polymerases and proof-reading exonucleases such as the ELONGASE Amplification System (GIBCO/BRL, Gaithersburg, Md.). Preferably, the process is automated with machines such as the Hamilton MICROLAB 2200 (Hamilton, Reno Nev.), Peltier thermal cycler (PTC200; MJ Research, Watertown, Mass.) and the ABI 377 DNA sequencers (Perkin Elmer). The nucleic acid sequences encoding *DevG20, DevG4, or DevG22* may be extended utilising a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the known region. The amplified sequences are

then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase. Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using OLIGO 4.06 primer analysis software (National Biosciences Inc., Plymouth, Minn.), or another appropriate program, to 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence temperatures about 68-72°C. The method uses several restriction enzymes to generate suitable fragments. The fragment is then circularised by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations also are used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

Another method which may be used to retrieve unknown sequences is that of Parker, J. D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries to walk in genomic DNA (Clontech, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences, which contain the 5' regions of genes. Use of a randomly primed library may be

especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions. Capillary electrophoresis systems, which are commercially available, may be used to analyse the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. GENOTYPER and SEQUENCE NAVIGATOR, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA, which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences which encode DevG20, DevG4, or DevG22 or fragments thereof, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of *DevG20, DevG4, or DevG22* in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences, which encode substantially the same, or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express *DevG20, DevG4, or DevG22*. As will be understood by those of skill in the art, it may be advantageous to produce *DevG20*-encoding, *DevG4*-encoding, and *DevG22*-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence. The nucleotide sequences of the present invention can

be engineered using methods generally known in the art in order to alter *DevG20, DevG4, or DevG22* encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding *DevG20, DevG4, or DevG22* may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of DevG20, DevG4, or DevG22 activities, it may be useful to produce chimeric proteins that can be recognised by a commercially available antibodies. A fusion protein may also be engineered to contain a cleavage site located between the DevG20, DevG4, or DevG22 encoding sequence and the heterologous protein sequences, so that DevG20, DevG4, or DevG22 may be cleaved and purified away from the heterologous moiety. In another embodiment, sequences encoding DevG20, DevG4, or DevG22 may be synthesised, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al. (1980) Nucl. Acids Res. Symp. Ser. 7:215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 7:225-232). Alternatively, the proteins themselves may be produced using chemical methods by synthesising the amino acid sequence, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A peptide synthesiser (Perkin Elmer). The newly synthesised peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and

Molecular Principles, WH Freeman and Co., New York, N.Y.). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequences, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active DevG20, DevG4, or DevG22, the nucleotide sequences coding therefor or functional equivalents, may be inserted into appropriate expression vectors, i.e., a vector, which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods, which are well known to those skilled in the art, may be used to construct expression vectors and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques. synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

A variety of expression vector/host systems may be utilised to contain and express sequences encoding DevG20, DevG4, or DevG22. These include, but are not limited to, micro-organisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or PBR322 plasmids); or animal cell systems. The "control elements" or "regulatory sequences" are those non-translated regions of the vector-enhancers, promoters, 5' and 3' untranslated regions

which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or PSPORT1 plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters and enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters and leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequences, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the protein. For example, when large quantities of protein are needed for the induction of antibodies, vectors, which direct high level expression of fusion proteins that are readily purified, may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as the BLUESCRIPT phagemid (Stratagene), in which the sequence encoding *DevG20, DevG4, or DevG22* may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. PGEX vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with Glutathione S-Transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins

made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will. In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al., (supra) and Grantet al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding *DevG20, DevG4, or DevG22* may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express *DevG20, DevG4, or DevG22*. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The sequences may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and place under control of the polyhedrin promoter. Successful insertions will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells of *Trichoplusia* larvae in which

DevG20, DevG4, or DevG22 may be expressed (Engelhard, E. K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilised. In cases where an adenovirus is used as an expression vector, sequences encoding *DevG20, DevG4, or DevG22* may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain viable viruses which are capable of expression in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding *DevG20, DevG4, or DevG22*, initiation codons, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein

in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express DevG20, DevG4, or DevG22 may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells, which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type. Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes, which can be employed in tk- or aprt-,cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to

chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilise indole in place of tryptophan, or hisD, which allows cells to utilise histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, ß- glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) Methods Mol. Biol. 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequences encoding *DevG20, DevG4, or DevG22* are inserted within a marker gene sequence, recombinant cells containing sequences encoding *DevG20, DevG4, or DevG22* can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with sequences encoding *DevG20, DevG4, or DevG22* under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well. Alternatively, host cells, which contain and express the nucleic acid sequences encoding *DevG20, DevG4, or DevG22*, may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA, or DNA-RNA hybridisation and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of polynucleotide sequences encoding *DevG20, DevG4, or DevG22* can be detected by DNA-DNA or DNA-RNA hybridisation or amplification using probes or portions or fragments of polynucleotides

specific for *DevG20, DevG4, or DevG22*. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences specific for *DevG20, DevG4, or DevG22* to detect transformants containing DNA or RNA encoding *DevG20, DevG4, or DevG22*. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of DevG20, DevG4, or DevG22, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes on the protein is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn.) and Maddox, D. E. et al. (1983; J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridisation or PCR probes for detecting sequences related to polynucleotides encoding *DevG20, DevG4, or DevG22* include oligo-labelling, nick translation, end-labelling or PCR amplification using a labelled nucleotide.

Alternatively, the sequences encoding *DevG20, DevG4, or DevG22*, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially

available, and may be used to synthesise RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, Mich.); Promega (Madison Wis.); and U.S. Biochemical Corp., (Cleveland, Ohio).

Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, co-factors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding *DevG20, DevG4, or DevG22* may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors may be designed to contain signal sequences, which direct secretion of proteins through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding DevG20, DevG4, or DevG22 to nucleotide sequences encoding a polypeptide domain, which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilised metals, protein A domains that allow purification on immobilised immunoglobulin, and the domain utilised in the FLAG extension/affinity purification system (Immunex Corp., Seattle, Wash.) The inclusion of cleavable linker sequences such as those specific for Factor XA or Enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the desired protein may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing DevG20, DevG4, or DevG22 and a nucleic acid encoding 6 histidine residues preceding a Thioredoxine or an

Enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilised metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3: 263-281)) while the Enterokinase cleavage site provides a means for purifying DevG20, DevG4, or DevG22 from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. et al. (1993; DNA Cell Biol. 12:441-453). In addition to recombinant production, fragments of DevG20, DevG4, or DevG22 may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A peptide synthesiser (Perkin Elmer). Various fragments of DevG20, DevG4, or DevG22 may be chemically synthesised separately and combined using chemical methods to produce the full length molecule.

Diagnostics and Therapeutics

The data disclosed in this invention show that the nucleic acids and proteins of the invention are useful in diagnostic and therapeutic applications implicated, for example but not limited to, in metabolic disorders like obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, pancreatic dysfunctions, arteriosclerosis, coronary artery disease (CAD), coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and other diseases and disorders. Hence, diagnostic and therapeutic uses for the DevG20, DevG4, or DevG22 proteins of the invention are, for example but not limited to, the following: (i) protein therapeutic, (ii) small molecule drug target, (iii) antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) diagnostic and/or prognostic marker, (v) gene therapy (gene

delivery/gene ablation), (vi) research tools, and (vii) tissue regeneration *in vitro* and *in vivo* (regeneration for all these tissues and cell types composing these tissues and cell types derived from these tissues).

The nucleic acids and proteins of the invention are useful in diagnositic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies and disorders. For example, but not limited to, cDNAs encoding the proteins of the invention and particularly their human homologues may be useful in gene therapy, and the DevG20, DevG4, or DevG22 proteins of the invention and particularly their human homologues may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from, for example, but not limited to, in metabolic disorders like obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and other diseases and disorders.

The nucleic acid encoding the DevG20, DevG4, or DevG22 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acids or the proteins are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

For example, in one aspect, antibodies which are specific for DevG20, DevG4, or DevG22 may be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express *DevG20, DevG4, or DevG22*. The antibodies

may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimerical, single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralising antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunised by injection with DevG20, DevG4, or DevG22 or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in human, BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum* are especially preferable. It is preferred that the peptides, fragments, or oligopeptides used to induce antibodies have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids. It is preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of amino acids may be fused with those of another protein such as keyhole limpet hemocyanin in order to enhance the immunogenicity.

Monoclonal antibodies to DevG20, DevG4, or DevG22 may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Köhler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods

81:31-42; Cote, R. J. et al. Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S. P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S. L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M. S. et al (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, D. R. (1991) Proc. Natl. Acad. Sci. 88:11120-3). Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments, which contain specific binding sites for DevG20, DevG4, or DevG22, may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by Pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W. D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding

and immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between the protein and its specific antibody. A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding *DevG20, DevG4, or DevG22*, or any fragment thereof, or antisense molecules, may be used for therapeutic purposes. In one aspect, antisense molecules may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding *DevG20, DevG4, or DevG22*. Thus, antisense molecules may be used to modulate protein activity, or to achieve regulation of gene function. Such technology is now well know in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding *DevG20, DevG4, or DevG22*. Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods, which are well known to those skilled in the art, can be used to construct recombinant vectors, which will express antisense molecules complementary to the polynucleotides of the gene encoding *DevG20, DevG4, or DevG22*. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra). Genes encoding *DevG20, DevG4, or DevG22* can be turned off by transforming a cell or tissue with expression vectors which express high levels of polynucleotide or fragment thereof which encodes *DevG20, DevG4, or DevG22*. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may

continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, e.g. DNA, RNA, or PNA molecules, to the control regions of the genes encoding *DevG20, DevG4, or DevG22*, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it cause inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J. E. et al. (1994) In; Huber, B. E. and B. I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, N.Y.). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyse the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridisation of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples, which may be used, include engineered hammerhead motif ribozyme molecules that can be specifically and efficiently catalyse endonucleolytic cleavage of sequences encoding *DevG20, DevG4, or DevG22*. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for

secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridisation with complementary oligonucleotides using ribonuclease protection assays.

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesising oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding *DevG20, DevG4, or DevG22*. Such DNA sequences may be incorporated into a variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesise antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues. RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognised by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use *in vivo*, *in vitro*, and *ex vivo*. For *ex vivo* therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods, which are well known in the art. Any of the therapeutic methods

described above may be applied to any suitable subject including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of DevG20, DevG4, or DevG22, antibodies to DevG20, DevG4, or DevG22, mimetics, agonists, antagonists, or inhibitors of DevG20, DevG4, or DevG22. The compositions may be administered alone or in combination with at least one other agent, such as stabilising compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones. The pharmaceutical compositions utilised in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which, can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.). Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as

tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including Arabic and tragacanth; and proteins such as gelatine and collagen. If desired, disintegrating or solubilising agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate. Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum Arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coating for product identification or to characterise the quantity of active compound, i.e., dosage. Pharmaceutical preparations, which can be used orally, include push-fit capsules made of gelatine, as well as soft, sealed capsules made of gelatine and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilisers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilisers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible

buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilisers or agents who increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilising processes. The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulphuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilised powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use. After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labelled for treatment of an indicated condition. For administration of DevG20, DevG4, or DevG22, such labelling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compounds, the therapeutically effective does can be estimated initially either in cell culture assays, e.g., of preadipocyte cell lines, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically effective dose refers to that amount of active ingredient, for example DevG20, DevG4, or DevG22 or fragments thereof, antibodies of DevG20, DevG4, or DevG22, to treat a specific condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions, which exhibit large therapeutic indices, are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage from employed, sensitivity of the patient, and the route of administration. The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors, which may be taken into account, include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy.

Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation. Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

In another embodiment, antibodies which specifically bind DevG20, DevG4, or DevG22 may be used for the diagnosis of conditions or diseases characterised by or associated with over- or underexpression of DevG20, DevG4, or DevG22, or in assays to monitor patients being treated with DevG20, DevG4, or DevG22, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays include methods, which utilise the antibody and a label to detect DevG20, DevG4, or DevG22 in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring DevG20, DevG4, or DevG22 are known in the art and provide a basis for diagnosing altered or abnormal levels of gene expression. Normal or standard values for expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibodies to DevG20, DevG4, or DevG22 under conditions suitable for complex formation. The amount of standard complex formation may be

quantified by various methods, but preferably by photometry, means. Quantities of DevG20, DevG4, or DevG22 expressed in control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides specific for *DevG20, DevG4, or DevG22* may be used for diagnostic purposes. The polynucleotides, which may be used, include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of *DevG20, DevG4, or DevG22* may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression, and to monitor regulation of gene expression levels during therapeutic intervention.

In one aspect, hybridisation with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding *DevG20, DevG4, or DevG22* or closely related molecules, may be used to identify nucleic acid sequences which encode *DevG20, DevG4, or DevG22*. The specificity of the probe, whether it is made from a highly specific region, e.g., unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridisation or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding *DevG20, DevG4, or DevG22*, alleles, or related sequences. Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the *DevG20, DevG4, or DevG22* encoding sequences. The hybridisation probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5 or from a genomic sequence including promoter,

enhancer elements, and introns of the naturally occurring *DevG20, DevG4, or DevG22* gene. Means for producing specific hybridisation probes for DNAs encoding *DevG20, DevG4, or DevG22* include the cloning of nucleic acid sequences encoding *DevG20, DevG4, or DevG22* or derivatives thereof into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesise RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labelled nucleotides. Hybridisation probes may be labelled by a variety of reporter groups, for example, radionuclides such as ^{32}P or ^{35}S, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences specific for *DevG20, DevG4, or DevG22* may be used for the diagnosis of conditions or diseases, which are associated with expression of *DevG20, DevG4, or DevG22*. Examples of such conditions or diseases include, but are not limited to, pancreatic diseases and disorders, including diabetes. Polynucleotide sequences specific for *DevG20, DevG4, or DevG22* may also be used to monitor the progress of patients receiving treatment for pancreatic diseases and disorders, including diabetes. The polynucleotide sequences may be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilising fluids or tissues from patient biopsies to detect altered gene expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding *DevG20, DevG4, or DevG22* may be useful in assays that detect activation or induction of various metabolic diseases and disorders, including obesity, as well as related disorders such as described above. The nucleotide sequences may be labelled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridisation complexes. After a suitable incubation period, the sample is washed and

the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample is significantly altered from that of a comparable have hybridised with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of disease associated with expression of *DevG20, DevG4, or DevG22*, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, which is specific for *DevG20, DevG4, or DevG22*, under conditions suitable for hybridisation or amplification. Standard hybridisation may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease. Once disease is established and a treatment protocol is initiated, hybridisation assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that, which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to metabolic diseases and disorders, including obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease,

hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the pancreatic diseases and disorders. Additional diagnostic uses for oligonucleotides designed from the sequences encoding *DevG20, DevG4, or DevG22* may involve the use of PCR. Such oligomers may be chemically synthesised, generated enzymatically, or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'.fwdarw.3') and another with antisense (3'.rarw.5'), employed under optimised conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of *DevG20, DevG4, or DevG22* include radiolabelling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P. C. et al. (1993) J. Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236). The speed of quantification of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantification.

In another embodiment of the invention, the nucleic acid sequences, which are specific for DevG20, DevG4, or DevG22, may also be used to generate

hybridisation probes, which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS, or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C. M. (1993) Blood Rev. 7:127-134, and Trask, B. J. (1991) Trends Genet. 7:149-154. FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, N.Y.) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding *DevG20, DevG4, or DevG22* on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help to delimit the region of DNA associated with that genetic disease.

The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals. *In situ* hybridisation of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R. A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The

nucleotide sequences of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, DevG20, DevG4, or DevG22, their catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds, e.g. peptides or low-molecular weight organic molecules, in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between the protein and the agent tested, may be measured.

Another technique for drug screening, which may be used, provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to DevG20, DevG4, or DevG22 large numbers of different test compounds, e.g. small molecules, are synthesised on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with the proteins, or fragments thereof, and washed. Bound proteins are then detected by methods well known in the art. Purified proteins can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralising antibodies can be used to capture the peptide and immobilise it on a solid support. In another embodiment, one may use competitive drug screening assays in which neutralising antibodies capable of binding DevG20, DevG4, or DevG22 specifically compete with a test compound for binding DevG20, DevG4, or DevG22. In this manner, the antibodies can be used to detect the presence of any peptide, which shares one or more antigenic determinants with DevG20, DevG4, or DevG22. In additional embodiments, the nucleotide sequences which encode *DevG20, DevG4, or DevG22* may be used in any molecular biology

techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The Figures show:

FIG. 1 shows the average increase of starvation resistance of HD-EP(X)10478 and HD-EP(X)31424 flies (Drosophila melanogaster; in the Figure, referred to as 10478 and 31424) by ecotopic expression using 'FB- or elav-Gal4 driver' (in comparison to wildtype flies (Oregon R); FB stands for fat body; elav-Gal4 stands for elevated Gal4). The average values for surviving flies (,average survivors) are given in % per time point (shown on the horizontal line as time of starvation; 8 hours (8h) to 72 hours (72 h)) are shown. See Examples for a more detailed description.

FIG. 2 shows the increase of triglyceride content of HD-EP(X)10478 and HD-EP(X)31424 flies by ectopic expression using "FB- or elav-Gal4 driver" (in comparison to wildtype flies (Oregon R)). Standard deviation of the measurements is shown as thin bars. Triglyceride content of the fly populations is shown in ug/mg wet weight (wt) of a fly (vertical).

FIG. 3 shows the molecular organisation of the *DevG20* locus.

FIG. 4A shows the nucleic acid sequence (SEQ ID NO:7) encoding the Drosophila DevG20 protein.

FIG. 4B shows the protein sequence (SEQ ID NO:8) of the Drosophila DevG20 encoded by the nucleic acid sequence shown in Figure 4A.

FIG. 4C shows the nucleic acid sequence (SEQ ID NO:1) of the human DevG20 homolog protein encoding the Homo sapiens hypothetical protein with Genbank Accession Number NM_030810.1 (MGC3178).

FIG. 4D shows the human DevG20 protein sequence (SEQ ID NO:2) (GenBank Accession Number NP_110437.1) encoded by the nucleic acid sequence shown in Figure 4C.

FIG. 5 shows the BLASTP (versus the non-redundant composite database) identity search result for Drosophila DevG20 protein (SEQ ID NO:8) and the human DevG20 protein (SEQ ID NO:2; GenBank Accession Number NP_110437.1), referred to as hG20 in the Figure. The middle sequence of the alignment shows identical amino acids in the one-letter code and conserved as +. Gaps in the alignment are represented as -.

FIG. 6 shows the expression of DevG20 in mammalian tissues.

FIG. 6A shows the real-time PCR analysis of DevG20-like expression in different wildtype mouse tissues. The relative RNA-expression is shown on the left hand side, the tissues tested are given on the horizontal line (for example, pancreas ('pancre'), white adipose tissue ('WA'), brown adipose tissue ('BA'), muscle ('muscl'), liver ('liv'), hypothalamus ('hypothalam'), cerebellum ('cerebell'), cortex ('corte'), midbrain ('midbra'); small intestine ('s. intestine'), heart ('hear'), lung ('lun'), spleen ('splee'), kidney ('kidne'), and bone marrow ('b. marrow').

FIG. 6B shows the real-time PCR analysis of DevG20-like expression in different mouse models (wildtype mice ('wt') – bars with light grey shading; fasted mice – bars with dark grey shading, obese mice ('ob/ob'), white bar) in different tissues (white adipose tissue ('WA'), brown adipose tissue ('BA'), muscle ('musc'), liver ('liv'), pancreas ('pancre'), hypothalamus ('hypothala'), cerebellum ('cerebell'), cortex ('corte'), midbrain ('midbra'); small intestine ('s. intestine'), heart ('hea'), lung ('lun'), spleen ('sple'), kidney ('kidn'), and bone marrow ('b. marrow').

FIG. 6C shows the real-time PCR analysis of DevG20-like expression during the differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes. The relative RNA-expression is shown on the left hand side,

the days of differention are shown on the horizontal line (d0 = day 0, start of the experiment, until d10 = day 10).

FIG. 7 shows the relative increase of triglyceride content of EP(2)0646, EP(2)2188, and EP(2)2517 flies caused by homozygous viable integration of the P-vector (in comparison to wildtype flies (EP-control)). Standard deviation of the measurements is shown as thin bars. Triglyceride content of the fly populations is shown as ration TG/Protein content.

FIG. 8 shows the molecular organisation of the *DevG4* gene locus.

FIG. 9A shows the nucleic acid sequence (SEQ ID NO:9) encoding the Drosophila DevG4 protein.

FIG. 9B shows the Drosophila DevG4 protein sequence (SEQ ID NO:10) encoded by the mRNA shown in Figure 9A.

FIG. 9C shows the nucleic acid sequence (SEQ ID NO:3) encoding the human DevG4 homolog (Homo sapiens ATP-binding cassette, sub-family C (CFTR/MRP), member 4, also referred to as ABCC4 and MPR4; GenBank Accession Number NM_005845).

FIG. 9D shows the protein sequence (SEQ ID NO:4; GenBank Accession Number NP_005836.1) of the human DevG4 homolog.

FIG. 10 shows protein domains (black boxes) of the human DevG4 protein.

FIG. 11 shows the comparison of DevG4 protein domains of different species (human ‚hMRP4', mouse (only shown in FIG. 11D, mMRP4), and Drosophila (DevG4)). Gaps in the alignment are represented as -. The alignment was produced using the multisequence alignment program of Clustal V software (Higgins,D.G. and Sharp,P.M. (1989). CABIOS, vol. 5, no. 2, 151-153.).

(A) Alignment of the ABC-membrane I domains. The identity of amino acids of Drosophila DevG4 and human DevG4 (hMRP4) is 41% and the similarity 63%.

(B) Alignment of the ABC-tran I domains. The identity of amino acids of Drosophila DevG4 and human DevG4 (hMRP4) is 56% and the similarity 75%.

(C) Alignment of the ABC-membrane II domains. The identity of amino acids of Drosophila DevG4 and human DevG4 (hMRP4) is 42% and the similarity 60%.

(D) Alignment of the ABC-tran II domains. The identity of amino acids of Drosophila DevG4 and human DevG4 (hMRP4) is 69% and the similarity 86%. Human and mouse ABC-tran II are almost identical.

FIG. 12 shows the expression of DevG4 in mammalian tissues.

FIG. 12A shows the real-time PCR analysis of DevG4 (MRP4) expression in different wildtype mouse tissues (pancreas ('pancre'), white adipose tissue ('WA'), brown adipose tissue ('BA'), muscle ('muscl'), liver ('liv'), hypothalamus ('hypothalam'), cerebellum ('cerebell'), cortex ('corte'), midbrain ('midbra'); small intestine ('s. intestine'), heart ('hear'), lung ('lun'), spleen ('splee'), kidney ('kidne'), and bone marrow ('b. marrow'). The relative RNA-expression is shown on the left hand side, the tissues tested are given on the horizontal line.

FIG. 12B shows the real-time PCR analysis of DevG4 (MRP4) expression in different mouse models (wildtype mice ('wt'), fasted mice, obese mice ('ob/ob')) in different tissues (white adipose tissue ('WA'), brown adipose tissue ('BA'), muscle ('muscl'), liver ('liv'), pancreas ('pancre'), hypothalamus ('hypothalam'), cerebellum ('cerebell'), cortex ('corte'), midbrain ('midbra'); small intestine ('s. intestine'), heart ('hear'), lung ('lun'), spleen ('splee'), kidney ('kidne'), and bone marrow ('b. marrow').

FIG. 12C shows the real-time PCR analysis of DevG4 (MRP4) expression during the differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes. The relative RNA-expression is shown on the left hand side,

the days of differention are shown on the horizontal line (d0 = day 0, start of the experiment, until d10 = day 10).

FIG. 13 shows the relative increase of triglyceride content of HD-EP(2)20388 and EP(2)2482 flies caused by homozygous viable integration of the P-vector (in comparison to wildtype flies (EP-control)). Standard deviation of the measurements is shown as thin bars. Triglyceride content of the fly populations is shown as ratio TG/Protein content in percent (%).

FIG. 14 shows the molecular organisation of the *DevG22* gene locus.

FIG. 15A shows the nucleic acid sequence (SEQ ID NO:11) encoding the Drosophila *DevG22* protein.

FIG. 15B shows the protein sequence (SEQ ID NO:12) of the Drosophila DevG22 protein.

FIG. 15C shows the nucleic acid sequence (SEQ ID NO:5) encoding the human DevG22 homolog (Homo sapiens ATP-binding cassette, sub-family G (WHITE), member 1 protein; GenBank Accession Number XM_009777).

FIG. 15D shows the protein sequence (SEQ ID NO:6) of the human DevG22 homolog (Homo sapiens ATP-binding cassette, sub-family G (WHITE), member 1 protein; GenBank Accession Number XP_009777.3).

FIG. 16 shows protein domain (black box) of the DevG22 protein.

FIG. 17 shows the alignment of human, mouse and fly DevG22 proteins (White-like ABC transporters). White-like ABC transporters only have a single ABC-tran protein domain. Drosophila DevG22 is 36% identical and 52% similar to human DevG22 (hWhite; ABC8, ABCG1, GenBank Accession Number XM_009777). Drosophila DevG22 is 36% identical and 51% similar to mouse DevG22 (mWhite; GenBank Accession Number

NP_033723). Human and mouse DevG22 proteins show 95% identity and 96% similarity.

FIG. 18 shows the expression of DevG22 in mammalian tissues.

FIG. 18A shows the real-time PCR analysis of DevG22 expression in different wildtype mouse tissues (pancreas ('pancre'), white adipose tissue ('WA'), brown adipose tissue ('BA'), muscle ('muscl'), liver ('liv'), hypothalamus ('hypothalam'), cerebellum ('cerebell'), cortex ('corte'), midbrain ('midbra'); small intestine ('sm. testine'), heart ('hear'), lung ('lun'), spleen ('splee'), kidney ('kidne'), and bone marrow ('b. marrow').

FIG. 18B shows the real-time PCR analysis of DevG22 expression in different mouse models (wildtype mice ('wt'), fasted mice, obese mice ('ob/ob')) in different tissues (white adipose tissue ('WA'), brown adipose tissue ('BA'), muscle ('muscl'), liver ('liv'), pancreas ('pancre'), hypothalamus ('hypothalam'), cerebellum ('cerebell'), cortex ('corte'), midbrain ('midbra'); small intestine ('s. intestine'), heart ('hear'), lung ('lun'), spleen ('splee'), kidney ('kidne'), and bone marrow ('b. marrow').

FIG. 18C shows the real-time PCR analysis of DevG22 expression during the differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes.

Examples

A better understanding of the present invention and of its many advantages will be evident from the following examples, only given by way of illustration.

Example 1: Isolation of EP-lines that have a novel function in energy homeostasis using a functional genetic screen

In order to isolate genes with a function in energy homeostasis several thousand EP-lines were crossed against two "Gal4-driver" lines that direct

expression of Gal4 in a tissue specific manner. Two different "driver"-lines were used in the screen: (i) expressing Gal4 mainly in the fatbody (FB), (ii) expressing Gal4 in neurons (elav) (FIGURE 1). After crossing the "driver"-line to the EP-line, an endogenous gene may be activated in fatbody or neurons respectively. For selection of relevant genes affecting energy homeostasis, the offspring of that cross was exposed to starvation conditions after six days of feeding. Wildtype flies show a constant starvation resistance. EP-lines with significantly changed starvation resistance were selected as positive candidates.

Example 2: HD-EP(X)10478 and HD-EP(X)31424 flies show significant starvation resistance when driven in the fatbody or neurons

Ectopic expression of the EP-lines HD-EP(X)10478 and HD-EP(X)31424, both homozygous viable integrations in the chromosomal region 10D4-10D6, under the control of the "FB-driver" and "elav-driver" caused a significant starvation resistance in comparison to wildtype flies (Oregon R, see FIGURE 1). Hundred flies offfspring of a cross or line were analysed under starvation conditions. Survivors per time point are shown in FIGURE 1. After 24 hours of starvation HD-EP(X)10478 and 31424 flies in combination with both "drivers" show 80-100% more survivors than the wildtype Oregon R. After 48 hours of starvation, almost no wildtype flies are still alive. In contrast, after 48 hours of starvation, about 20% of the population of HD-EP(X)10478 and 31424 in combination with both "drivers" are alive which is a significant increase. Few flies of HD-EP(X)10478 and 31424 in combination with both "drivers" still survive after 72 hours of starvation where normally no wildtype flies are alive. Therefore, ectopic expression via HD-EP(X)10478 and HD-EP(X)31424 in the fatbody and neurons of *Drosophila melanogaster* leads to significant starvation resistance.

Example 3: Triglyceride content is increased by ectopic expression via HD-EP(X)10478 and HD-EP(X)31424 in the fatbody and weaker in the neurons

Starvation resistance can have its origin due to changes in energy homeostasis, e.g., reduction of energy consumption and/or increase in storage of substances like triglycerides. Triglycerides are the most efficient storage for energy in cells. Therefore the content of triglycerides of a pool of flies with the same genotype was analysed using an triglyceride assay.

For determination of triglyceride content of flies, several aliquots of each time ten females of HD-EP(X)10478 and HD-EP(X)31424, HD-EP(X)10478/FB-Gal4 and HD-EP(X)31424/FB-Gal4, HD-EP(X)10478/elav-Gal4 and HD-EP(X)31424/elav-Gal4 and Oregon R were analysed. Flies were incubated for 5 min at 90°C in an aqueous buffer using a waterbath, followed by hot extraction. After another 5 min incuabation at 90°C and mild centrifugation, the triglyceride content of the flies extract was determined using Sigma Triglyceride (INT 336-10 or –20) assay by measuring changes in the optical density according to the manufacturer's protocol. As a reference fly mass was measured on a fine balance before extraction procedure.

The result of the triglyceride contents analysis is shown in FIGURE 2. The average increase of triglyceride content of HD-EP(X)10478 and 31424 flies in combination with the "FB- and elav Gal4-driver" lines is shown in comparison to wildtype flies (Oregon R) and the HD-EP(X)10478 and 31424 integrations alone. Standard deviations of the measurments are shown as thin bars. Triglyceride content of the different fly populations is shown in µg/mg wet weight (wt.) of a fly. In each assay ten females of the offspring of a cross or line were analysed in the triglyceride assay after feeding that offspring for six days. The assay was repeated several times. Wildtype flies show a constant triglyceride level of 30 to 45 µg/mg wet weight of a fly. HD-EP(X)10478 and 31424 flies show a similar or slightly

lower triglyceride content than wildtype. In contrast, HD-EP(X)10478 and 31424 flies in combination with both "drivers" show an average increase up to 1.8-fold of 55 to 72 µg/mg wet wt. in comparison to wildtype (Oregon R)flies and the HD-EP(X)10478 and 31424 integration alone. Therefore, gain of a gene activity in the locus 10D4-6 is responsible for changes in the metabolism of the energy storage triglycerides.

Ectopic expression of genomic *Drosophila* sequences using FB-Gal4 caused an average 1.8-fold increase of triglyceride content in comparison to wildtype flies (Oregon R). HD-EP(X)10478 and HD-EP(X)31424 under the control of the "elav-Gal4-driver" caused a weaker increase of triglyceride content. Therefore ectopic expression via HD-EP(X)10478 and HD-EP(X)31424 in the fatbody of *Drosophila melanogaster* leads to a significant increase of the energy storage triglyceride and therefore represents an obese fly model. The increase of triglyceride content by gain of a gene function suggests a gene activity in energy homeostasis in a dose dependent and tissue specific manner that controls the amount of energy stored as triglycerides.

Example 4: Measurement of triglyceride content of homozygous flies (EP(2)0646, EP(2)2188, EP(2)2517, HD-EP(2)20388, EP(2)2482)

Triglycerides are the most efficient storage for energy in cells. In order to isolate genes with a function in energy homeostasis, several thousand EP-lines were tested for their triglyceride content after a prolonged feeding period. Lines with significantly changed triglyceride content were selected as positive candidates for further analysis. In this invention, the content of triglycerides of a pool of flies with the same genotype after feeding for six days was analysed using a triglyceride assay. For determination of triglyceride content, several aliquots of each time 10 males of the offspring of a cross or line were analysed after feeding the offspring for six days. Fly mass was measured on a fine balance as a reference. Flies were extracted

in methanol/chloroform (1:1) and an aliquot of the extract was evaporated under vacuum. Lipids were emulsified in an aqueous buffer with help of sonification. Triglyceride content was determined using Sigma INT 336-10 or -20 assay by measuring changes in the optical density according to the manufacturer's protocol.

Improving and simplifying the determination of triglyceride content of flies, In each assay ten males of the offspring of a cross or line were analysed in the triglyceride assay after feeding that offspring for six days; the assay was repeated several times. Flies were incubated for 5 min at 90°C in an aqueous buffer using a waterbath, followed by hot extraction. After another 5 min incubation at 90°C and mild centrifugation, the triglyceride content of the flies extract was determined using Sigma Triglyceride (INT 336-10 or -20) assay by measuring changes in the optical density according to the manufacturer's protocol. As a reference protein content of the same extract was measured using BIO-RAD *DC* Protein Assay according to the manufacturer's protocol.

Wildtype flies show constantly a triglyceride level of 11 to 23 µg/mg wet weight of a fly. EP(2)0646, EP(2)2188, EP(2)2517, and HD-EP(2)20388, and EP(2)2482 homozygous flies show constantly a higher triglyceride content than the wildtype (FIGURES 7 and 13). In contrast, EP(2)0646, EP(2)2188, EP(2)2517, HD-EP(2)20388, and EP(2)2482 flies in combination with both "drivers" show sometimes only a slightly increase (2.1- to 2.3-fold of 49 to 53 µg/mg wet wt) in comparison to the wildtype (Oregon R) (not shown). Therefore, the loss of gene activity in the loci, where the P-vector of EP(2)0646, EP(2)2188, EP(2)2517, HD-EP(2)20388, and EP(2)2482 flies is homozygous viably integrated, is responsible for changes in the metabolism of the energy storage triglycerides, therefore representing in both cases an obese fly model. The increase of triglyceride content due to the loss of a gene function suggests potential gene

activities in energy homeostasis in a dose dependent manner that controls the amount of energy stored as triglycerides.

Example 5: Identificiation of the genes

DevG20 (PDI)

Nucleic acids encoding the DevG20 protein of the present invention were identified using plasmid-rescue technique. Genomic DNA sequences of about 1 kb were isolated that are localised directly 3' to HD-EP(X)10478 or HD-EP(X)31424 integrations. Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly) were screened thereby confirming the integration side of HD-EP(X)10478 and HD-EP(X)31424 and nearby localised endogenous genes (FIGURE 3). Figure 3 shows the molecular organisation of the DevG20 locus. Genomic DNA sequence is represented by the assembly AE003487 as a black line that includes the integration sites of EP(X)1503, HD-EP(X)10478 and HD-EP(X)31424. Numbers represent the coordinates of AE003487 genomic DNA, the predicted genes and the EP-vector integration sites. Arrows represent the direction of ectopic expression of endogenous genes controlled by the Gal4 promoters in the EP-vectors. Predicted exons of genes CG2446 and CG1837 are shown as grey bars. Using plasmid rescue method about 1 kb genomic DNA sequences that are directly localised 3' of the HD-EP(X)10478 and HD-EP(X)31424 integration sites were isolated. Using the 1 kb plasmid rescue DNA public DNA sequence databases were screened thereby identifying the integration sites of HD-EP(X)10478 and HD-EP(X)31424.

HD-EP(X)10478 and HD-EP(X)31424 are integrated in the predicted gene CG2446 that is represented by the EST clots 241_2-4 but their Gal4 promoters direct ectopic expression of endogenous genes in the opposite direction in respect to the direction of CG2446 expression. About 2 kb 3' of HD-EP(X)10478 and HD-EP(X)31424 integration sites the predicted gene

CG1837 is localized that corresponds to est clot 3553_14 and could be expressed ectopically using "FB- and elav-Gal4-drivers". The ectopic expression of CG1837 in the fatbody or weaker in neurons leads to increase of triglyceride content in flies.

HD-EP(X)10478 is inserted into the first predicted exon of CG2446 that corresponds to the EST clot 241_2-4 in antisense orientation. Gal4 promoter region of HD-EP(X)10478 drives expression in the opposite direction than CG2446 is expressed therefore could drive the ectopic expression of another endogenous gene. HD-EP(X)31424 is inserted in the first predicted exon of CG2446 and its Gal4 promoter drives expression in the opposite direction in comparison to CG2446 expression. A different endogenous gene CG1837 corresponding to EST clot 3553_14 is localized 2180 base pairs 3' in sense direction of both EP-integrations. CG1837 can be expressed ectopically via HD-EP(X)10478 and HD-EP(X)31424, leading to obesity.

DevG4 (MRP4)

Nucleic acids encoding the DevG4 protein of the present invention were identified using plasmid-rescue technique. Genomic DNA sequences of about 0.8 kb were isolated that are localised directly 3' to the EP(2)0646, EP(2)2517 and EP(2)2188 integration. Using those isolated genomic sequences public databases like Berkeley *Drosophila* Genome Project (GadFly) were screened thereby confirming the integration side of 0646, EP(2)2517 and EP(2)2188 and nearby localised endogenous genes (FIGURE 8). FIGURE 8 shows the molecular organisation of the *DevG4* locus. In Figure 8, genomic DNA sequence is represented by the assembly as a dotted black line (17.5 kb, starting at position 8256000 on chromosome 2L) that includes the integration sites of 0646, EP(2)2517 and EP(2)2188 (arrows). Numbers represent the coordinates of the genomic DNA. Arrows represent the direction of ectopic expression of endogenous genes controlled by the Gal4 promoters in the EP-vectors. Transcribed DNA

sequences (ESTs and clots) are shown as bars in the lower two lines. Predicted exons of gene CG7627 (GadFly) are shown as green bars and introns as grey bars.

0646, EP(2)2517 and EP(2)2188 are integrated directly 5' of the EST Clot 6022_1 in antisense orientation. Clot 6022_1 represents a cDNA clone meaning that is showing that the DNA sequence is expressed in *Drosophila*. Clot 6022_1 sequence overlaps with the sequence of the predicted gene CG7627 therefore Clot 6022_1 includes the 5' end of *DevG4* gene and EP(2)0646 and EP(2)2517 are homozygous viably integrated in the promoter of *DevG4*. Using the 0.8 kb plasmid rescue DNA, public DNA sequence databases were screened thereby identifying the integration sites of EP(2)0646 and EP(2)2517. It was found that EP(2)0646 and EP(2)2517 are integrated in the promoter of the gene with GadFly Accession Number CG7627 that is also represented by the EST clot 6022_1. The Gal4 promoters of should direct ectopic expression of endogenous genes in the opposite direction in respect to the direction of CG7627 expression. Therefore, expression of the CG7627 could be effected by homozygous viable integration of EP(2)0646, EP(2)2517 and EP(2)2188 leading to increase of the energy storage triglycerides

DevG22

FIGURE 14 shows genomic DNA sequence represented by the assembly as a dotted black line (15 kb, starting at position 171400.5 on chromosome 2L) that includes the integration site of EP(2)20388 (arrow). Numbers represent the coordinates of the genomic DNA. Arrows represent the direction of ectopic expression of endogenous genes controlled by the Gal4 promoters in the EP-vectors. Transcribed DNA sequences (ESTs and clots) are shown as green bars in another line. Predicted exons of gene with GadFly Accession Number CG17646 are shown as green bars and introns as grey bars. It was found that *DevG22* encodes for a novel gene that is predicted by GadFly sequence analysis programs as CG17646. Using

plasmid rescue method about 0.6 kb genomic DNA sequences that are directly localised 3' of the EP(2)20388 integration site were isolated. Using the 0.6 kb plasmid rescue DNA, public DNA sequence databases were screened thereby identifying the integration site of EP(2)20388. EP(2)20388 is integrated directly 5' of the EST SD03967 in sense orientation. SD03967 represents a cDNA clone meaning that its DNA sequence is expressed in *Drosophila*. SD03967 sequence overlaps with the 5' sequence of the predicted gene CG17646 therefore SD03967 includes the 5' and the 3' end of *DevG22* gene. The 3' end of SD03967 does not overlap with CG17646 sequence therefore the cDNA of *DevG22* might be even longer than shown in FIGURE 14. EP(2)20388 is integrated in the promoter of the gene CG17646 that is also represented by EST SD03967; its Gal4 promoter should direct ectopic expression of CG17646. Therefore, expression of the CG17646 could be effected by homozygous viable integration of EP(2)20388 leading to increase of the energy storage triglycerides.

Example 6: Analysis of DevG20

DevG20 encodes for a novel gene that is predicted by GadFly sequence analysis programs and isolated EST clones. Neither phenotypic nor functional data are available in the prior art for the novel gene CG1837, referred to as *DevG20* in the present invention. The present invention is describing the nucleic acid sequence of *DevG20*, as shown in FIGURE 4A, SEQ ID NO:1.

The present invention is describing a polypeptide comprising the amino acid sequence of SEQ ID NO:2, as presented using the one-letter code in FIGURE 4B. DevG20 is 416 amino acids in length. An open reading frame was identified by beginning with an ATP initiation codon at nucleotide 37 and ending with a CAC stop codon at nucleotide 1284 (FIG. 4B).

The predicted amino acid sequence was searched in the publicly available GenBank database. In search of sequence databases, it was found, for example, that DevG20 has 60% homology with human hGRP58 protein, a potential 58 kDa glucose regulated protein of 324 amino acids (GenBank Accession Number NP_110437.1; identical to former Accession Numbers AAH01199 and BC001199) (see FIGURE 4C and 4D; SEQ ID NO:1 and 2). In particular, Drosophila DevG20 and human hGRP58 protein share 60% homology (see FIG. 5), starting between amino acid 84 and 407 of DevG20 (and amino acids 1 to 316 of hGRP58). hGRP58 protein is homologous to a mouse protein encoded by the cDNA clone 601333564F1 NCI_CGAP_Mam6, identified using tblastp sequence comparison of a protein with translated mouse EST clones.

Using InterPro protein analysis tools, it was found, for example, that the DevG20 protein has at least three Thioredoxin protein motifs and an endoplasmic reticulum target sequence. These motifs and targeting sequencing are also found in glucose-regulated proteins and Protein disulfide isomerases. Glucose regulated proteins and Protein disulfide isomerases are chaperones that are involved in many different processes like lipoprotein assembly at the endoplasmic reticulum.

DevG20 encodes for a novel protein that is homologous to the family of protein disulfide isomerases or glucose regulated proteins. Based upon homology, DevG20 protein of the invention and each homologous protein or peptide may share at least some activity.

Example 7: Analysis of *DevG4*

As described above, DevG4 is encoded by GadFly Accession Number CG7627. The nucleic acid sequence of Drosophila *DevG4*, as shown in FIGURE 9A, SEQ ID NO:9. The present invention is describing a polypeptide comprising the amino acid sequence of SEQ ID NO:10, as

presented using the one-letter code in FIGURE 9B. Drosophila DevG4 protein is 1355 amino acids in length. An open reading frame was identified beginning with an ATP initiation codon at nucleotide 158 and ending with a stop codon at nucleotide 4225. Drosophila DevG4 has additional 28 amino acids at the N-terminus without changing the frame in comparison to the predicted CG7627 protein after combining Clot 6022_1 and CG7627 cDNA sequences.

The predicted amino acid sequence was searched in the publicly available GenBank (NCBI) database. The search indicated, that Drosophila DevG4 has about 40% identity with human MRP4 (MOAT-B) protein, a ATP-binding cassette (ABC) transporter protein of 1325 amino acids (Accession Number: NP_005836; SEQ ID NO:10) (see FIGURE 9C). In particular, Drosophila DevG4 and human homolog DevG4 (hMRP4) proteins share about 80% homology (see FIGURE 9D), starting between amino acid 8 and 1330 of DevG4 (and amino acids 7 to 1277 of hMRP4).

Since the protein domains found in member of the ABC superfamily are highly conserved, a comparison (Clustal X 1.8) between the four protein domains of Drosophila DevG4 with human and mouse homolog proteins was conducted (see FIGURE 11). We found that human and mouse (sequence is only partially available) MRP4 as closest homologous proteins to the Drosophila DevG4 protein. Using InterPro protein analysis tools, it was found, that the DevG4 protein has at least 4 four protein motifs domains (FIGURE 10). These motifs and targeting sequencing are found throughout the whole ABC transporter superfamliy. ABC transporters are membrane spanning proteins that are involved in many different transport processes. FIGURE 11 A shows the alignment of the ABC-membrane I domains. The identity of amino acids of Drosophila DevG4 and human hMRP4 is 41% and the similarity of the sequence is 63%. Figure 11 B shows the alignment of the ABC-tran I domains. The identity of amino acids of Drosophila DevG4 and human hMRP4is 56% and the similarity

75%. No mouse sequence is available. Figure 11 C shows the alignment of the ABC-membrane II domains. The identity of amino acids of Drosophila DevG4 and human hMRP4 is 42% and the similarity 60%. No mouse sequence is available. Figure 11 D shows the alignment of the ABC-tran II domains. No mouse sequence is available. The identity of amino acids of Drosophila DevG4 and human hMRP4is 69% and the similarity 86%. Human and mouse ABC-tran II domains are almost identical.

Based upon homology, Drosophila DevG4 protein and each homologous protein or peptide may share at least some activity. The DevG4 protein has two characteristic ABC-membrane domains, a six transmembrane helical region (labeled 'ABC_membrane' in FIG 10, ABC transporter transmembrane region) which anchors the protein in cell membranes. In addition, DevG4 has two ABC-transporter domains of several hundred amino acid residues (labeled 'ABC-tran' in FIGURE 10, ABC transporter), including an ATP-binding site. Proteins of the ABC family are membrane spanning proteins associated with a variety of distinct biological processes in both prokaryotes and eukaryotes, for example in transport processes such as active transport of small hydrophilic molecules across the cytoplasmic membrane. Furthermore, a single MMR-HSR1 domain (GTPase of unknown function, light grey square box in FIG. 4A) was identified in DevG4. FIGURE 10 shows the has a single characteristic ABC-transporter domain ('ABC trans') of the DevG22 protein.

Example 8: Analyis of DevG22

As discussed above, Drosophila DevG22 protein is encoded GadFly accession number CG17646. The present invention is describing the nucleic acid sequence of *DevG22*, as shown in FIGURE 15A, SEQ ID NO:11. The present invention is describing a polypeptide comprising the amino acid sequence of SEQ ID NO:12, as presented using the one-letter code in FIGURE 15B. Drosophila DevG22 protein is 627 amino acids in

length. An open reading frame was identified beginning with an ATP initiation codon at nucleotide 576 and ending with a stop codon at nucleotide 2459.

The predicted amino acid sequence was searched in the publicly available GenBank database. In search of sequence databases, it was found, for example, that DevG22 has almost 40% identity with human White (ABC8, ABCG1) protein, a ATP-binding cassette (ABC) transporter protein of 674 amino acids (GenBank Accession Number XP_009777.3; see FIGURE 15C and 15D; SEQ ID NO:5 and SEQ ID NO:6). In particular, Drosophila DevG22 and the human homolog protein share about 70% homology (see FIG. 17), starting between amino acid 57 and 622 of Drosophila DevG22 (and amino acids 27 to 507 of human DevG22-hWhite).

Using InterPro protein analysis tools, it was found that the DevG22 protein has at least 1 one protein motif (FIGURE 16). The White-like subfamily of ABC transporters is characterized by the single ABC-tran domain and the overall amino acid sequence. Therefore, the complete coding sequence and not only the domains are compared. Figure 17 shows the alignment of human, mouse, and Drosophila DevG22 proteins. Drosophila DevG22 is 36% identical and 52% similar to human DevG22 (hWhite; ABC8, ABCG1, GenBank Accession Number XP_009777). Drosophila DevG22 is 36% identical and 51% similar to mouse DevG22 (mWhite; GenBank Accession Number NP_033723). Therefore, the vertebrate white transporter is the closest homologue to Drosophila DevG22. Human and mouse White proteins show 95% identity and 96% similarity. Based upon homology, DevG22 protein of the invention and each homologous protein or peptide may share at least some activity.

Example 9: Expression of the polypeptides in mammalian tissues

For analyzing the expression of the polypeptides disclosed in this invention in mammalian tissues, several mouse strains (preferrably mice strains C57Bl/6J, C57Bl/6 ob/ob and C57Bl/KS db/db which are standard model systems in obesity and diabetes research) were purchased from Harlan Winkelmann (33178 Borchen, Germany) and maintained under constant temperature (preferrably 22°C), 40 per cent humidity and a light / dark cycle of preferrably 14 / 10 hours. The mice were fed a standard chow (for example, from ssniff Spezialitäten GmbH, order number ssniff M-Z V1126-000). Animals were sacrificed at an age of 6 to 8 weeks. The animal tissues were isolated according to standard procedures known to those skilled in the art, snap frozen in liquid nitrogen and stored at –80°C until needed.

For analyzing the role of the proteins disclosed in this invention in the *in vitro* differentiation of different mammalian cell culture cells for the conversion of pre-adipocytes to adipocytes, mammalian fibroblast (3T3-L1) cells (e.g., Green & Kehinde, Cell 1: 113-116, 1974) were obtained from the American Tissue Culture Collection (ATCC, Hanassas, VA, USA; ATCC-CL 173). 3T3-L1 cells were maintained as fibroblasts and differentiated into adipocytes as described in the prior art (e.g., Qiu. et al., J. Biol. Chem. 276:11988-95, 2001; Slieker et al., BBRC 251: 225-9, 1998). At various time points of the differentiation procedure, beginning with day 0 (day of confluence) and day 2 (hormone addition; for example, dexamethason and 3-isobutyl-1-methylxanthin), up to 10 days of differentiation, suitable aliquots of cells were taken every two days. Alternatively, mammalian fibroblast 3T3-F442A cells (e.g., Green & Kehinde, Cell 7: 105-113, 1976) were obtained from the Harvard Medical School, Department of Cell Biology (Boston, MA, USA). 3T3-F442A cells were maintained as fibroblasts and differentiated into adipocytes as described previously (Djian, P. et al., J. Cell. Physiol., 124:554-556, 1985). At various time points of

the differentiation procedure, beginning with day 0 (day of confluence and hormone addition, for example, Insulin), up to 10 days of differentiation, suitable aliquots of cells were taken every two days. 3T3-F442A cells are differentiating in vitro already in the confluent stage after hormone (insulin) addition.

RNA was isolated from mouse tissues or cell culture cells using Trizol Reagent (for example, from Invitrogen, Karlsruhe, Germany) and further purified with the RNeasy Kit (for example, from Qiagen, Germany) in combination with an DNase-treatment according to the instructions of the manufacturers and as known to those skilled in the art. Total RNA was reverse transcribed (preferrably using Superscript II RNaseH$^-$ Reverse Transcriptase, from Invitrogen, Karlsruhe, Germany) and subjected to Taqman analysis preferrably using the Taqman 2xPCR Master Mix (from Applied Biosystems, Weiterstadt, Germany; the Mix contains according to the Manufacturer for example AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, passive reference Rox and optimized buffer components) on a GeneAmp 5700 Sequence Detection System (from Applied Biosystems, Weiterstadt, Germany).

For the analysis of the expression of DevG20, taqman analysis was performed using the following primer/probe pair (see FIGURE 6): Mouse DevG20 (PDI) forward primer (SEQ ID NO:13): 5'-CAC GGG TGA CAA GGG CA-3'; mouse DevG20 (PDI) reverse primer (SEQ ID NO:14): 5'-CCC CTG TGC AAT AGT GTC CTC-3'; Taqman probe (SEQ ID NO:15): (5/6-FAM) TGC TGG CAC TCA CCG AGA AGA GCT T (5/6-TAMRA).

As shown in Figure 6A, real time PCR (Taqman) analysis of the expression of DevG20 protein in mammalian (mouse) tissues revealed that DevG20 (PDI) is rather ubiquitously expressed in various mouse tissues. However, a clear expression in WAT and BAT can also be demonstrated. DevG20 (PDI) shows an up-regulation of its expression in BAT, cortex and spleen of

genetically obese ob/ob mice (FIGURE 6B). In addition, its expression in kidney and bone marrow of fasted mice is also up-regulated. Even though no up-regulation of DevG20 (PDI) expression in WAT of ob/ob mice has been observed, we can clearly demonstrate a two-fold up-regulation of its expression during the in vitro differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes (FIGURE 6C).

For the analysis of the expression of DevG4, taqman analysis was performed using the following primer/probe pair (see FIGURE 12): Mouse DevG4 (mrp4) forward primer (SEQ ID NO:16): 5'-CAA GTA GCG CCC ACC CC-3'; Mouse DevG4 (mrp4) reverse primer (SEQ ID NO:17): 5'-AGT TCA CAT TGT CGA AGA CGA TGA-3'; Taqman probe (SEQ ID NO:18): (5/6-FAM) AGG CTG GCC CCA CGA GGG A (5/6-TAMRA).

Taqman analysis revealed that DevG4 (mrp4) is ubiquitously expressed in various mouse tissues with highest levels of expression found in kidney (FIGURE 12A). DevG4 (mrp4) shows a very prominent up-regulation of its expression in liver of genetically obese ob/ob mice (FIGURE 12B). In addition, a significant up-regulation in kidney can also be observed under these conditions. Under fasting conditions, DevG4 (mrp4) expression seems to show a global down-regulation of its expression, this is especially prominent in the BAT tissue of fasting mice. DevG4 (mrp4) expression increases approximately 4-fold during the in vitro differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes (FIGURE 12C).

For the analysis of the expression of DevG22, taqman analysis was performed using the following primer/probe pair: Mouse DevG22 (white) forward primer (SEQ ID NO:18): 5'-TCG TAT ACT GGA TGA CGT CCC A-3'; Mouse DevG22 (white) reverse primer (SEQ ID NO:19): 5'-TGG TAC CCA GAG CAG CGA AC-3'; Taqman probe (SEQ ID NO:20): (5/6-FAM) CCG TCG GAC GCT GTG CGT TTT (5/6-TAMRA).

Taqman analysis revealed that DevG22 (white) is predominantly expressed in neuronal tissues. However, a clear expression in other tissues like WAT or BAT has also been noted (FIGURE 18A and 18B). The expression of DevG22 (white) in BAT and WAT is under metabolic control: In fasted mice, expression goes up in BAT. Contrary to this, expression is increased in WAT and muscle in genetically obese ob/ob mice (FIGURE 18B). This up-regulation in ob/ob mice correlates with the observed strong up-regulation of DevG22 (white) expression during the in vitro differentiation of 3T3-L1 cells (FIGURE 18C).

All publications and patents mentioned in the above specification are herein incorporated by reference.

Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Literature

Klucken J, Büchler C, Orsó E, Kaminski WE, Porsch-Özcürümez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M, Allikmets R, Schmitz G.: ABCG1 (ABC8), the human homolog of the *Drosophila* white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci USA. 2000 Jan;97(2):817-22.

Orsó E, Broccardo C, Kaminski WE, Böttcher A, Liebisch G, Drobnik W, Götz A, Chambenoit O, Diederich W, Langmann T, Spruss T, Luciani M-F, Rothe G, Lackner KJ, Chimini G, Schmitz G.: Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and Abc1-deficient mice. Nat Genet. 2000 Feb;24:192-6.

Venkateswaran A, Repa JJ, Lobaccaro J-MA, Bronson A, Mangelsdorf DJ, Edwards PA.: Human White/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. J Biol Chem. 2000 May;275(19):14700-7.

Nakamura M, Ueno S, Sano A, Tanabe H.: Polymorphisms of the human homologue of the *Drosophila* white gene are associated with mood and panic disorders. Mol Psychiatry. 1999 Mar;4(2):155-62.

Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G.: The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet. 1999 Aug;22(4):347-51.

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ,

Hayden MR, et al.: Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet. 1999 Aug;22(4):336-45.

Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G.: Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet. 1999 Aug;22(4):352-5.

Schuetz JD, Connelly MC, Sun D, Paibir SD, Flynn PM, Srinivas RV, Kumar A, Fridland A.: MRP4. A previously unidentified factor in resistance to nucleoside-based antiviral drugs. Nat Med. 1999 Sep;5(9):1048-51.

Wada M, Toh S, Taniguchi K, Nakamura T, Uchiumi T, Kohno K, Yoshida I, Kimura A, Sakisaka S, Adachi Y, Kuwano M.: Mutations in the canilicular organic anion transporter (cMOAT) gene, a novel ABC transporter, in patients with hyperbilirubinemia II/Dubin-Johnson syndrome. Hum Mol Genet. 1998 Feb;7(2):203-7.

Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR.: A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet. 1997 Mar;15(3):236-46.

Claims

1. A pharmaceutical composition comprising a nucleic acid molecule of the protein disulfide isomerase (DevG20) or ABC transporter (DevG4, DevG22) gene family or a polypeptide encoded thereby or a fragment or a variant of said nucleic acid molecule or said polypeptide or an antibody, an aptamer or another receptor recognizing a nucleic acid molecule of the protein disulfide isomerase or ABC transporter gene family or a polypeptide encoded thereby together with pharmaceutically acceptable carriers, diluents and/or adjuvants.

2. The composition of claim 1, wherein the nucleic acid molecule is a vertebrate or insect protein disulfide isomerase or ABC transporter nucleic acid, particularly a human protein disulfide isomerase nucleic acid (DevG20)(Genbank Accession No. NM_030810, SEQ ID NO:1) or an ABC transporter nucleic acid such as the human MRP4 nucleic acid (DevG4)(Genbank Accession No. NM_005845; SEQ ID NO:3) or the human White nucleic acid (DevG22)(Genbank Accession No. XM_009777; SEQ ID NO:5) or a fragment thereof or a variant thereof and/or a nucleic acid molecule complementary thereto.

3. The composition of claim 1 or 2, wherein said nucleic acid molecule
   (a) hybridizes at 50°C in a solution containing 0.2 x SSC and 0.1% SDS to a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 1, 3 or 5 and/or the complementary strand thereof;
   (b) it is degenerate with respect to the nucleic acid molecule of (a);
   (c) encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at

(c) encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at least 98% and up to 99,6% identical to SEQ ID NO: 2, 4 or 6;

(d) differs from the nucleic acid molecule of (a) to (g) by mutation and wherein said mutation causes an alteration, deletion, duplication or premature stop in the encoded polypeptide.

4. The composition of any one of claims 1-3, wherein the nucleic acid molecule is a DNA molecule, particularly a cDNA or a genomic DNA.

5. The composition of any one of claims 1-4, wherein said nucleic acid encodes a polypeptide contributing to regulating the engergy homeostasis and the metabolism of triglycerides.

6. The composition of any one of claims 1-5, wherein said nucleic acid molecule is a recombinant nucleic acid molecule.

7. The composition of any one of claims 1-6, wherein the nucleic acid molecule is a vector, particularly an expression vector.

8. The composition of any one of claims 1-5, wherein the polypeptide is a recombinant polypeptide.

9. The composition of claim 8, wherein said recombinant polypeptide is a fusion polypeptide.

10. The composition of any one of claims 1-7, wherein said nucleic acid molecule is selected from hybridization probes, primers and anti-sense oligonucleotides.

11. The composition of any one of claims 1-10 which is a diagnostic composition.

12. The composition of any one of claims 1-10 which is a pharmaceutical composition.

13. The composition of any one of claims 1-12 for the manufacture of an agent for detecting and/or verifying, for the treatment, alleviation and/or prevention of an disorder, such as obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, pancreatic dysfunctions, arteriosclerosis, coronary artery disease (CAD), coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, disorders related to ROS production, and neurodegenerative diseases, and others, in cells, cell masses, organs and/or subjects.

14. Use of a nucleic acid molecule of the DevG20, DevG4, and/or DevG22 gene family or a polypeptide encoded thereby or a fragment or a variant of said nucleic acid molecule or said polypeptide or an antibody, an aptamer or another receptor recognizing said nucleic acid molecule or a polypeptide encoded thereby for controlling the function of a gene and/or a gene product which is influenced and/or modified by a Dev620, Dev64 and/or Dev622 polypeptide.

15. Use of a nucleic acid molecule of the DevG20, DevG4, and/or DevG22 gene family or a polypeptide encoded thereby or a fragment or a variant of said nucleic acid molecule or said polypeptide or an antibody, an aptamer or another receptor recognizing said nucleic acid molecule or a polypeptide encoded thereby for identifying substances capable of interacting with a DevG20, DevG4, and/or DevG22 polypeptide.

16. A non-human transgenic animal exhibiting a modified expression of a DevG20, DevG4, and/or DevG22 polypeptide.

17. The animal of claim 16, wherein the expression of the DevG20, DevG4, and/or DevG22 polypeptide is increased and/or reduced.

18. A recombinant host cell exhibiting a modified expression of a DevG20, DevG4, and/or DevG22 polypeptide.

19. The cell of claim 18 which is a human cell.

20. A method of identifying a polypeptide involved in the regulation of energy homeostasis and/or metabolism of triglycerides in a mammal comprising the steps of

(a) contacting a collection of (poly)peptides with a DevG20, DevG4, and/or DevG22 polypeptide or a fragment thereof under conditions that allow binding of said (poly)peptides;

(b) removing (poly)peptides which do not bind; and

(c) identifying (poly)peptides that bind to said DevG20, DevG4, and/or DevG22 polypeptide.

21. A method of screening for an agent which modulates the interaction of a DevG20, DevG4, and/or DevG22 polypeptide with a binding target/agent, comprising the steps of

(a) incubating a mixture comprising

(aa) a DevG20, DevG4, and/or DevG22 polypeptide, or a fragment thereof;

(ab) a binding target/agent of said DevG20, DevG4, and/or DevG22 polypeptide or fragment thereof; and

(ac) a candidate agent

under conditions whereby said DevG20, DevG4, and / or DevG22 polypeptide or fragment thereof specifically binds to said binding target/agent at a reference affinity;

(b) detecting the binding affinity of said DevG20, DevG4, and/or DevG22 polypeptide or fragment thereof to said binding target to determine an (candidate) agent-biased affinity; and

(c) determining a difference between (candidate) agent-biased affinity and the reference affinity.

22. A method of producing a composition comprising the (poly)peptide identified by the method of claim 20 or the agent identified by the method of claim 21 with a pharmaceutically acceptable carrier, diluent and/or adjuvant.

23. The method of claim 22 wherein said composition is a pharmaceutical composition for preventing, alleviating or treating obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and other diseases and disorders.

24. Use of a polypeptide as identified by the method of claim 20 or of an agent as identified by the method of claim 21 for the preparation of a pharmaceutical composition for the treatment, alleviation and/or prevention of obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, arteriosclerosis, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea.

25. Use of a nucleic acid molecule of the DevG20, DevG4, and/or DevG22 family or of a fragment thereof for the preparation of a non-human animal which over- or underexpresses the DevG20, DevG4, and/or DevG22 gene product.

26. Kit comprising at least one of

(a) a DevG20, DevG4, and/or DevG22 nucleic acid molecule or a fragment thereof;

(b) a vector comprising the nucleic acid of (a);

(c) a host cell comprising the nucleic acid of (a) or the vector of (b);

(d) a polypeptide encoded by the nucleic acid of (a);

(e) a fusion polypeptide encoded by the nucleic acid of (a);

(f) an antibody, an aptamer or another receptor against the nucleic acid of (a) or the polypeptide of (d) or (e); and

(g) an anti-sense oligonucleotide of the nucleic acid of (a).

FIGURE 1: Increase of starvation resistance of HD-EP(X)10478 and HD-EP(X)31424 flies by expression using "FB- or elav-Gal4 driver"

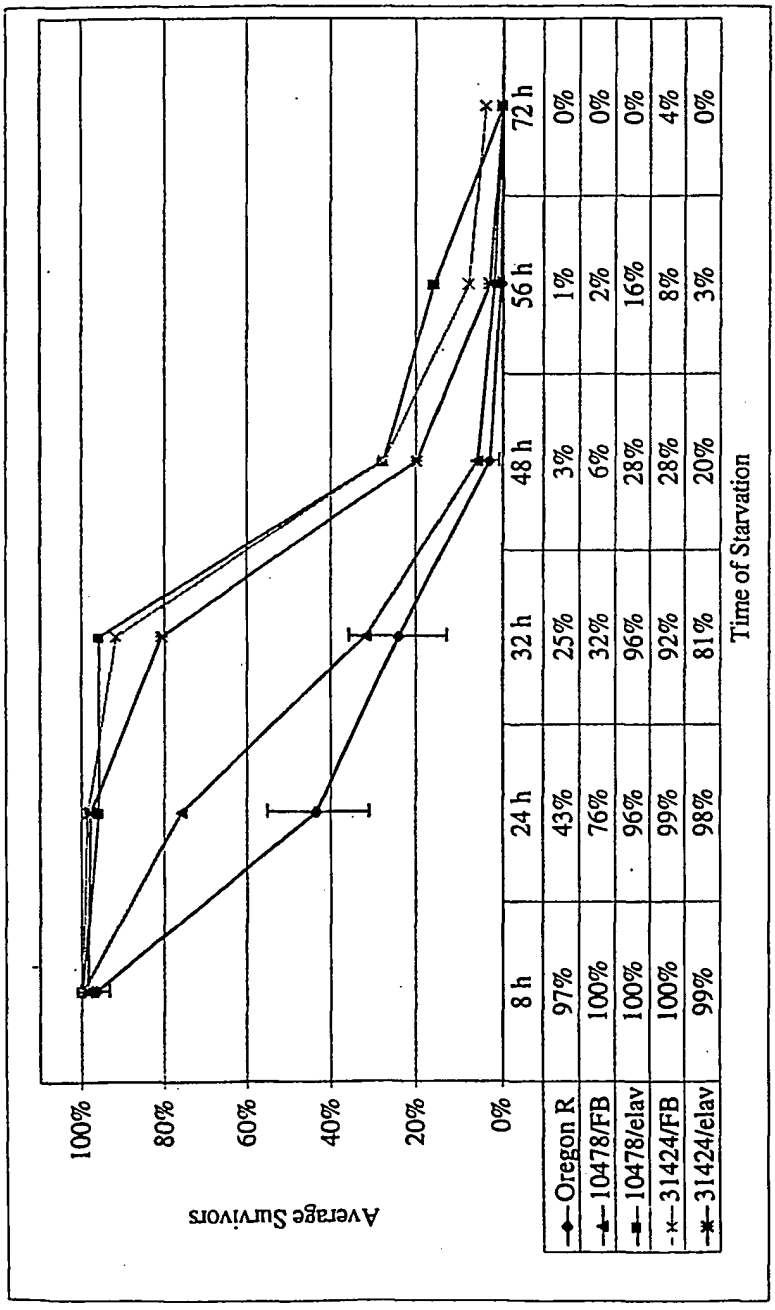


FIGURE 2: Increase of triglyceride content of HD-EP(X)10478 and 31424 flies by ectopic expression using "FB- or elav-Gal4 driver"

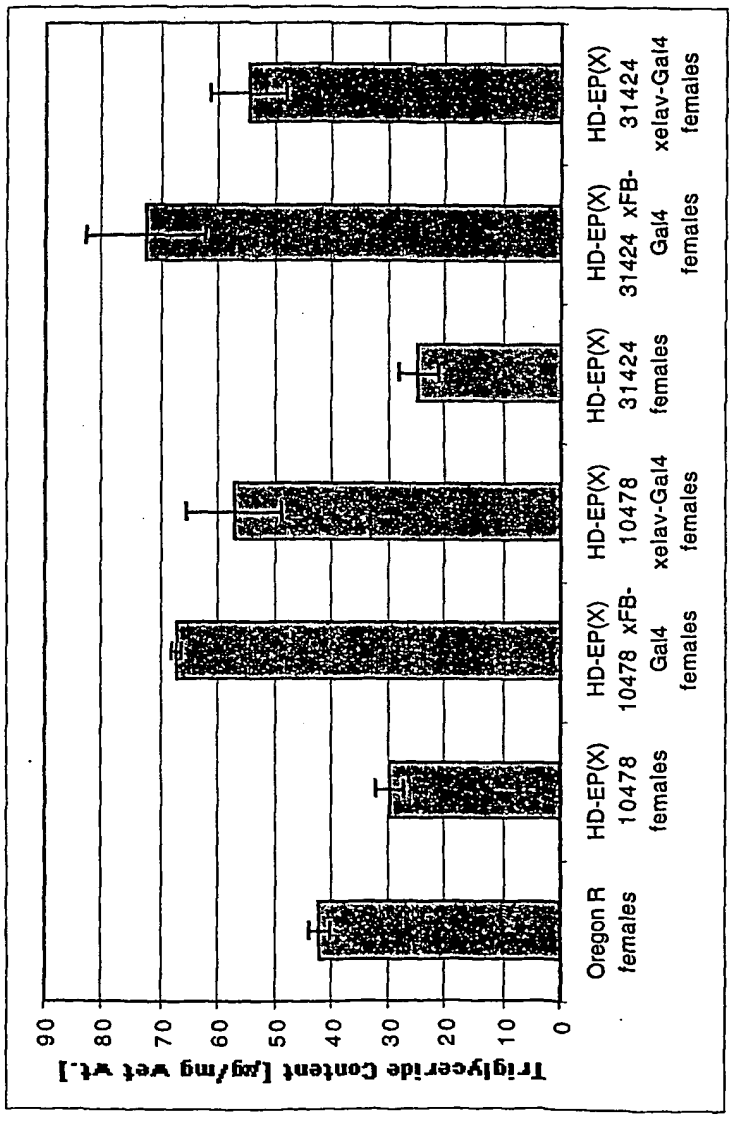


FIGURE 3: Molecular organisation of the *DevG20* locus

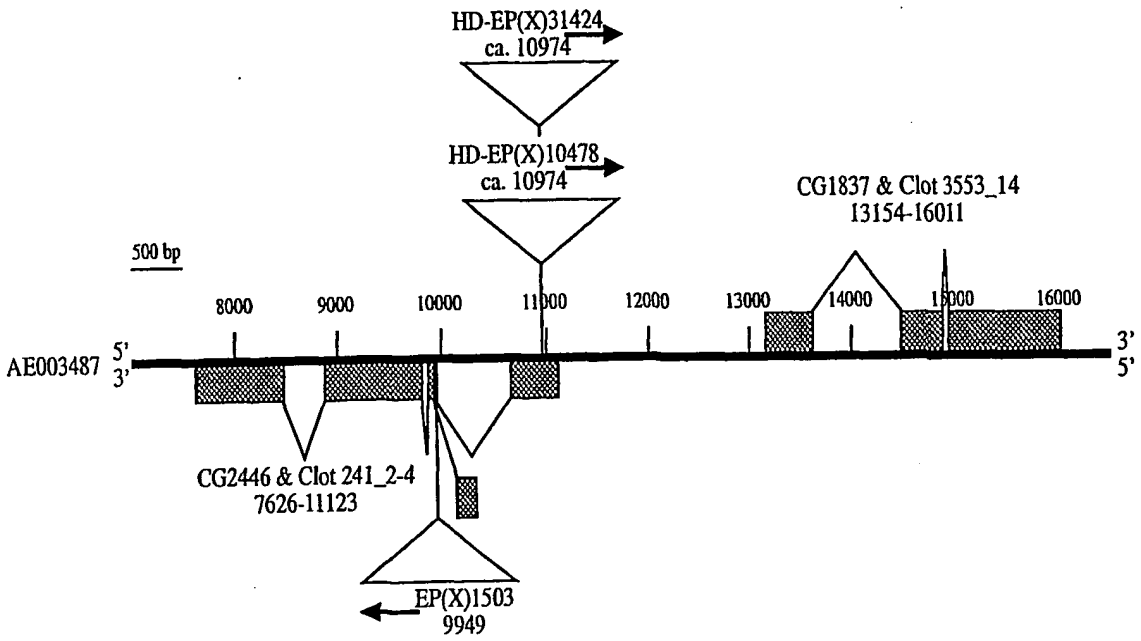


FIGURE 4A: Nucleotide sequence of the *DevG20* cDNA (SEQ ID NO:7)

CAACACTTTCAGATCTCTCCAAGCGTTCCGTGCATCATGTTGACCAGGTCCATTTTATCCGTTGCCGTGTGTGGCCTCCTCCTGTCG
CCCCTCCTGCCCATCACCCGTGCGTCCCAGGAGGAGGACACCGGCAAACAGGACAAGCAGTTCACCGTGGAACTGGACCCAGAAACC
TTCGACACTGCGATTGCCGGCGGCAATGTCTTCGTCAAGTTCTTTGCTCCATGGTGCGGCCATTGCAAGCGTATTCAGCCGCTGTGG
GAACAGCTGGCCGAGATCATGAATGTGGATAACCCCAAGGTGATCATCGCCAAGGTGGACTGCACCAAGCACCAGGGACTCTGCGCC
ACGCACCAGGTTACCGGCTATCCCACACTGCGCCTCTTTAAGCTGGGCGAAGAGGAATCGGTCAAGTTTAAGGGCACTCGCGACTTG
CCCGCCATTACCGATTTCATCAATAAGGAACTGAGCGCACCCGCTGAGGCGGATCTGGGCGAGGTCAAGCGCGAGCAGGTCGAGAAC
CTTAATATTGGCAAGGTGGTTGACCTTACCGAAGACACCTTTGCCAAGCACGTGTCCACCGGCAATCACTTTGTCAAATTCTTTGCA
CCCTGGTGCAGTCATTGTCAGCGTTTGGCACCCACGTGGGAGGACCTGGCCAAGGAGCTGATTAAAGAGCCTACCGTAACTATCTCG
AAGATCGACTGCACCCAGTTCCGTTCCATCTGCCAGGACTTTGAGGTCAAGGGGTATCCCACTCTTCTCTGGATCGAGGATGGCAAA
AAGATTGAAAAGTACTCGGGTGCTCGCGATCTGTCCACGCTGAAAACGTACGTGGAGAAAATGGTGGGCGTGCCACTGGAGAAGACC
GCTGGCGAGGCCGGCGATGAGAAGGTGGTTATCGAGGAGGTTGCCGGCGAGGAGGACGCAGCCAAGAAGCTGACTCCACAACAGCTG
ACTGGCGAGGACGAGTTCGACCAAGCCATTGCCGAGGGCGTTGCCTTCATTAAGTTCTATGCTCCGTGGTGTGGACACTGCCAGAAG
CTGCAACCCACCTGGGAGCAGCTGGCCACGGAAACGCACCAGGCTCAGAGTTCCGTGAAAATTGCCAAGGTTGACTGCACGGCGCCG
GAGAACAAGCAAGTGTGCATCGACCAGCAGGTGGAGGGCTATCCAACTCTCTTCCTTTACAAGAATGGTCAGCGCCAGAACGAGTAC
GAAGGCAGCCGCTCACTGCCGGAGCTGCAGGCCTATCTGAAGAAGTTCCTCGGCCACGACGAGCTCTAAAGCATCTGCCGGTTCACA
GGGAATCAGCAGTAGTCGAATGATCAAATAATCGCCCGCCAAATTAATTGTAATCGTAATTTTTCTTTCTCCAGCATAAACCTCTGT
GAGGAGTCCGCATTTAATCAATATATCCAACAACACCCAAACGACAACGCGCTAGCTGAAATTGATAAATTCAATCTGAGTTCCTTT
TACTTTTTTTTCGTTTTTGTCCCTCTTCCTTAGCTGTTTTTGTAGAATTTTTTTTATACTTATTTGTTTAGTGTTTACGTAAATGGG
GCACGGGACTGTGTGCGCGATGATAAGCCATTTAGCGAACTTACATTTCAATTTTAAAGTCATTTTGAGTAGCCTACGTTTTAGGAA
AATTTGTCAACACAAAGCTAATAAATATCAATTGGAAAAC

FIGURE 4B: Amino acid sequence of *DevG20* protein (SEQ ID NO:8)

MLTRSILSVAVCGLLLSPLLPITRASQEEDTGKQDKQFTVELDPETFDTAIAGGNVFVKFFAPWCGHCKRIQPLWEQLAEIMNVDNP
KVIIAKVDCTKHQGLCATHQVTGYPTLRLFKLGEEESVKFKGTRDLPAITDFINKELSAPAEADLGEVKREQVENLNIGKVVDLTED
TFAKHVSTGNHFVKFFAPWCSHCQRLAPTWEDLAKELIKEPTVTISKIDCTQFRSICQDFEVKGYPTLLWIEDGKKIEKYSGARDLS
TLKTYVEKMVGVPLEKTAGEAGDEKVVIEEVAGEEDAAKKLTPQQLTGEDEFDQAIAEGVAFIKFYAPWCGHCQKLQPTWEQLATET
HQAQSSVKIAKVDCTAPENKQVCIDQQVEGYPTLFLYKNGQRQNEYEGSRSLPELQAYLKKFLGHDEL

FIGURE 4C: Nucleotide sequence of the human Dev20 homolog (SEQ ID NO:1)

GTCATGTTCT TCGCGCCCTG GTGTGGACAC TGCCAGCGGC TGCAGCCGAC TTGGAATGAC CTGGGAGACA
AATACAACAG CATGGAAGAT GCCAAAGTCT ATGTGGCTAA AGTGGACTGC ACGGCCCACT CCGACGTGTG
CTCCGCCCAG GGGGTGCGAG GATACCCCAC CTTAAAGCTT TTCAAGCCAG GCCAAGAAGC TGTGAAGTAC
CAGGGTCCTC GGGACTTCCA GACACTGGAA AACTGGATGC TGCAGACACT GAACGAGGAG CCAGTGACAC
CAGAGCCGGA AGTGGAACCG CCCAGTGCCC CCGAGCTCAA GCAAGGGCTG TATGAGCTCT CAGCAAGCAA
CTTTGAGCTG CACGTTGCAC AAGGCGACCA CTTTATCAAG TTCTTCGCTC CGTGGTGTGG TCACTGCAAA
GCCCTGGCTC CAACCTGGGA GCAGCTGGCT CTGGGCCTTG AACATTCCGA AACTGTCAAG ATTGGCAAGG
TTGATTGTAC ACAGCACTAT GAACTCTGCT CCGGAAACCA GGTTCGTGGC TATCCCACTC TTCTCTGGTT
CCGAGATGGG AAAAAGGTGG ATCAGTACAA GGGAAAGCGG GATTTGGAGT CACTGAGGGA GTACGTGGAG
TCGCAGCTGC AGCGCACAGA GACTGGAGCG ACGGAGACCG TCACGCCCTC AGAGGCCCCG GTGCTGGCAG
CTGAGCCCGA GGCTGACAAG GGCACTGTGT TGGCACTCAC TGAAAATAAC TTCGATGACA CCATTGCAGA
AGGAATAACC TTCATCAAGT TTTATGCTCC ATGGTGTGGT CATTGTAAGA CTCTGGCTCC TACTTGGGAG
GAACTCTCTA AAAAGGAATT CCCTGGTCTG GCGGGGGTCA AGATCGCCGA AGTAGACTGC ACTGCTGAAC
GGAATATCTG CAGCAAGTAT TCGGTACGAG GCTACCCCAC GTTATTGCTT TTCCGAGGAG GGAAGAAAGT
CAGTGAGCAC AGTGGAGGCA GAGACCTTGA CTCGTTACAC CGCTTTGTCC TGAGCCAAGC GAAAGACGAA
CTTTAGGAAC ACAGTTGGAG GTCACCTCTC CTGCCCAGCT CCCGCACCCT GCGTTTAGGA GTTCAGTCCC
ACAGAGGCCA CTGGGTTCCC AGTGGTGGCT GTTCAGAAAG CAGAACATAC TAAGCGTGAG GTATCTTCTT
TGTGTGTGTG TTTTCCAAGC CAACACACTC TACAGATTCT TTATTAAATG TGTAACTCAT GGTCACTGTG
TAAACATTTT CAGTGGCGAT ATATCCCCTT TGACCTTCTC TTGATGAAAT TTACATGGTT TCCTTTGAGA
CTAAAATAGC GTTGAGGGAA ATGAAATTGC TGGACTATTT GTGGCTCCTG AGTTGAGTGA TTTTGGTGAA
AGAAAGCACA TCCAAAGCAT AGTTTACCTG CCCACGAGTT CTGGAAAGGT GGCCTTGTGG CAGTATTGAC
GTTCCTCTGA TCTTAAGGTC ACAGTTGACT CAATACTGTG TTGGTCCGTA GCATGGAGCA GATTGAAATG
CAAAAACCCA CACCTCTGGA AGATACCTTC ACGGCCGCTG CTGGAGCTTC TGTTGCTGTG AATACTTCTC
TCAGTGTGAG AGGTTAGCCG TGATGAAAGC AGCGTTACTT CTGACCGTGC CTGAGTAAGA GAATGCTGAT
GCCATAACTT TATGTGTCGA TACTTGTCAA ATCAGTTACT GTTCAGGGGA TCCTTCTGTT TCTCACGGGG
TGAAACATGT CTTTAGTTCC TCATGTTAAC ACGAAGCCAG AGCCCACATG AACTGTTGGA TGTCTTCCTT
AGAAAGGGTA GGCATGGAAA ATTCCACGAG GCTCATTCTC AGTATCTCAT TAACTCATTG AAAGATTCCA

GTTGTATTTG TCACCTGGGG TGACAAGACC AGACAGGCTT TCCCAGGCCT GGGTATCCAG GGAGGCTCTG
CAGCCCTGCT GAAGGGCCCT AACTAGAGTT CTAGAGTTTC TGATTCTGTT TCTCAGTAGT CCTTTTAGAG
GCTTGCTATA CTTGGTCTGC TTCAAGGAGG TCGACCTTCT AATGTATGAA GAATGGGATG CATTTGATCT
CAAGACCAAA GACAGATGTC AGTGGGCTGC TCTGGCCCTG GTGTGCACGG CTGTGGCAGC TGTTGATGCC
AGTGTCCTCT AACTCATGCT GTCCTTGTGA TTAAACACCT CTATCTCCCT TGGGAATAAG CACATACAGG
CTTAAGCTCT AAGATAGATA GGTGTTTGTC CTTTTACCAT CGAGCTACTT CCCATAATAA CCACTTTGCA
TCCAACACTC TTCACCCACC TCCCATACGC AAGGGGATGT GGATACTTGG CCCAAAGTAA CTGGTGGTAG
GAATCTTAGA AACAAGACCA CTTATACTGT CTGTCTGAGG CAGAAGATAA CAGCAGCATC TCGACCAGCC
TCTGCCTTAA AGGAAATCTT TATTAATCAC GTATGGTTCA CAGATAATTC TTTTTTTAAA AAAACCCAAC
CTCCTAGAGA AGCACAACTG TCAAGAGTCT TGTACACACA ACTTCAGCTT TGCATCACGA GTCTTGTATT
CCAAGAAAAT CAAAGTGGTA CAATTTGTTT GTTTACACTA TGATACTTTC TAAATAAACT CTTTTTTTTT
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AC

FIGURE 4D: Amino acid sequence of the human Dev20 homolog (SEQ ID NO:2)

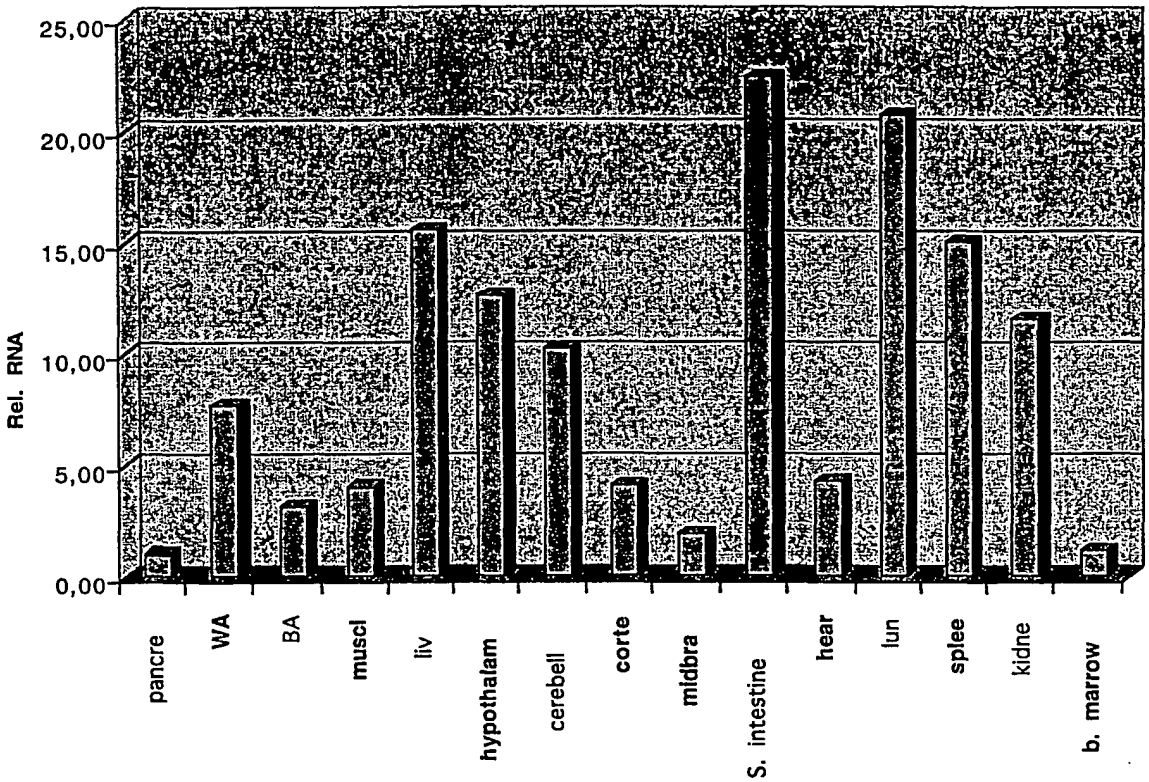
MEDAKVYVAK VDCTAHSDVC SAQGVRGYPT LKLFKPGQEA VKYQGPRDFQ TLENWMLQTL NEEPVTPEPE
VEPPSAPELK QGLYELSASN FELHVAQGDH FIKFFAPWCG HCKALAPTWE QLALGLEHSE TVKIGKVDCT
QHYELCSGNQ VRGYPTLLWF RDGKKVDQYK GKRDLESLRE YVESQLQRTE TGATETVTPS EAPVLAAEPE
ADKGTVLALT ENNFDDTIAE GITFIKFYAP WCGHCKTLAP TWEELSKKEF PGLAGVKIAE VDCTAERNIC
SKYSVRGYPT LLLFRGGKKV SEHSGGRDLD SLHRFVLSQA KDEL

FIGURE 5: Sequence alignment (BLASTP) of the DevG20 protein with the human homolog (hDevG20; SEQ ID NO:2)

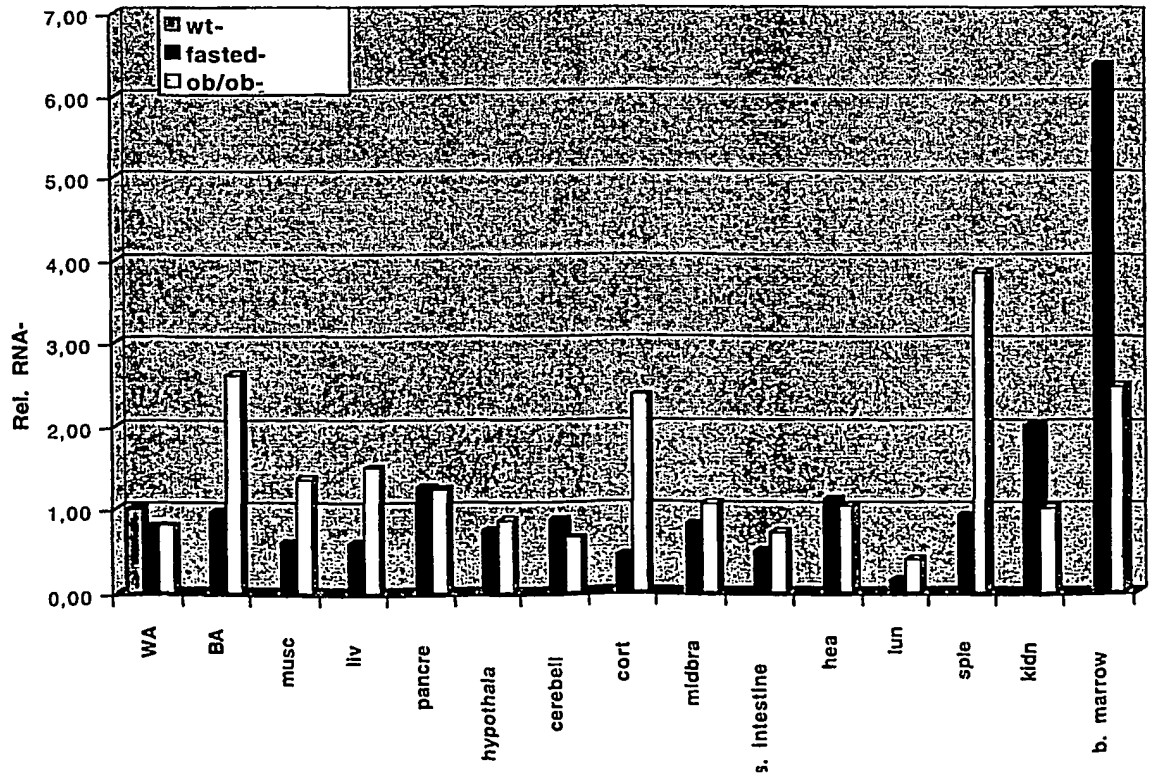
```
Score = 267 bits (684), Expect = 2e-70
Identities = 146/325 (44%), Positives = 200/325 (60%), Gaps = 10/325 (3%)

DevG20:  84  VDNPKVIIAKVDCTKHQGLCATHQVTGYPTLRLFKLGEEESVKFKGTRDLPAFTDFINKE 143
             +++ KV +AKVDCT H  +C+   V GYPTL+LFK G +E+VK++G RD   + +++ +
hDevG20: 1   MEDAKVYVAKVDCTAHSDVCSAQGVRGYPTLKLFKPG-QEAVKYQGPRDFQTLENWMLQT 59

DevG20:  144 LSAPAEADLGEVKREQVENLNIGKVVDLTEDTFAKHVSTGNHFVKFFAPWCSHCQRLAPT 203
             L+        EV+      L  G + +L+   F  HV+ G+HF+KFFAPWC HC+ LAPT
hDevG20: 60  LNEEPVTPEPEVEPPSAPELKQG-LYELSASNFELHVAQGDHFIKFFAPWCGHCKALAPT 118

DevG20:  204 WEDLAKELIKEPTVTISKIDCTQFRSICQDFEVKGYPTLLWIEDGKKIEKYSGARDLSTL 263
             WE LA  L    TV I K+DCTQ   +C   +V+GYPTLLW  DGKK+++Y G RDL +L
hDevG20: 119 WEQLALGLEHSETVKIGKVDCTQHYELCSGNQVRGYPTLLWFRDGKKVDQYKGKRDLESL 178

DevG20:  264 KTYVEKMVGVPLEKTAGEAGDEKVVIEE-VAGEEDAAKKLTPQQLTGEDEFDQAIAEGVA 322
             + YVE      L++T   A +     E  V   E  A K T   LT E+ FD  IAEG+
hDevG20: 179 REYVESQ----LQRTETGATETVTPSEAPVLAAEPEADKGTVLALT-ENNFDDTIAEGIT 233

DevG20:  323 FIKFYAPWCGHCQKLQPTWEQLATETHQAQSSVKIAKVDCTAPENKQVCIDQQVEGYPTL 382
             FIKFYAPWCGHC+ L PTWE+L+ +      + VKIA+VDCTA  N  +C    V GYPTL
hDevG20: 234 FIKFYAPWCGHCKTLAPTWEELSKKEFPGLAGVKIAEVDCTAERN--ICSKYSVRGYPTL 291

DevG20:  383 FLYKNGQRQNEYEGSRSLPELQAYL 407
              L++ G++ +E+ G R L  L  ++
hDevG20: 292 LLFRGGKKVSEHSGGRDLDSLHRFV 316
```

FIGURE 6: Expression of DevG20 in mammalian tissues

(A) Real-time PCR analysis of DevG20 expression in *wildtype mouse tissues*



(B) Real-time PCR mediated comparison of DevG20 expression in different mouse models



(C) Real-time PCR mediated comparison of DevG20 expression during the differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes

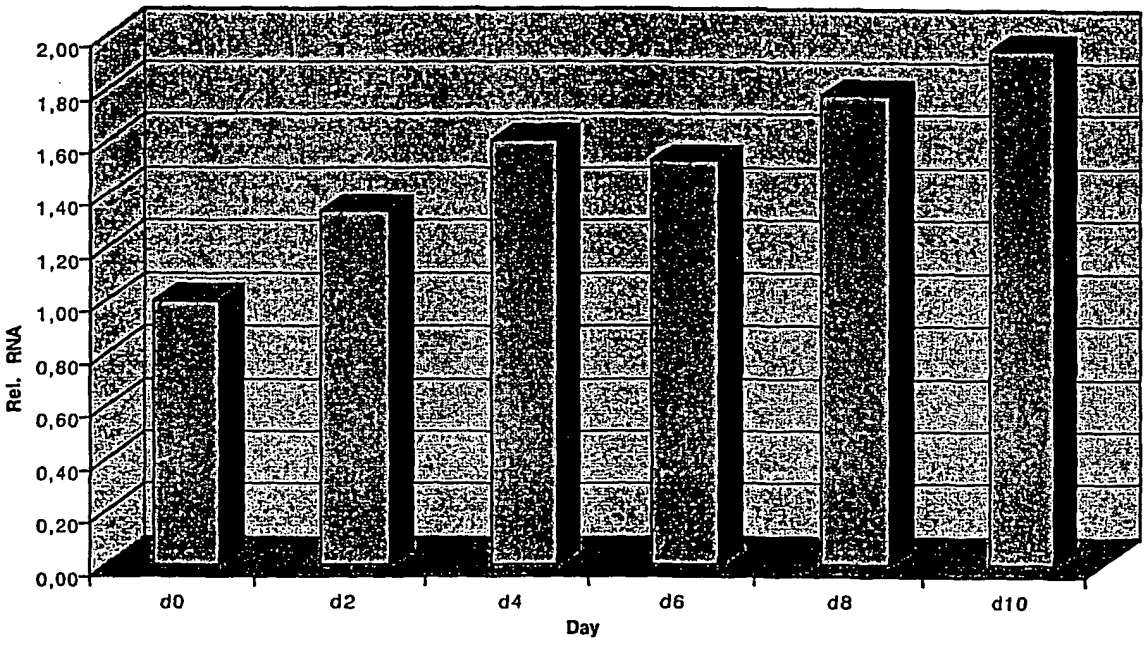
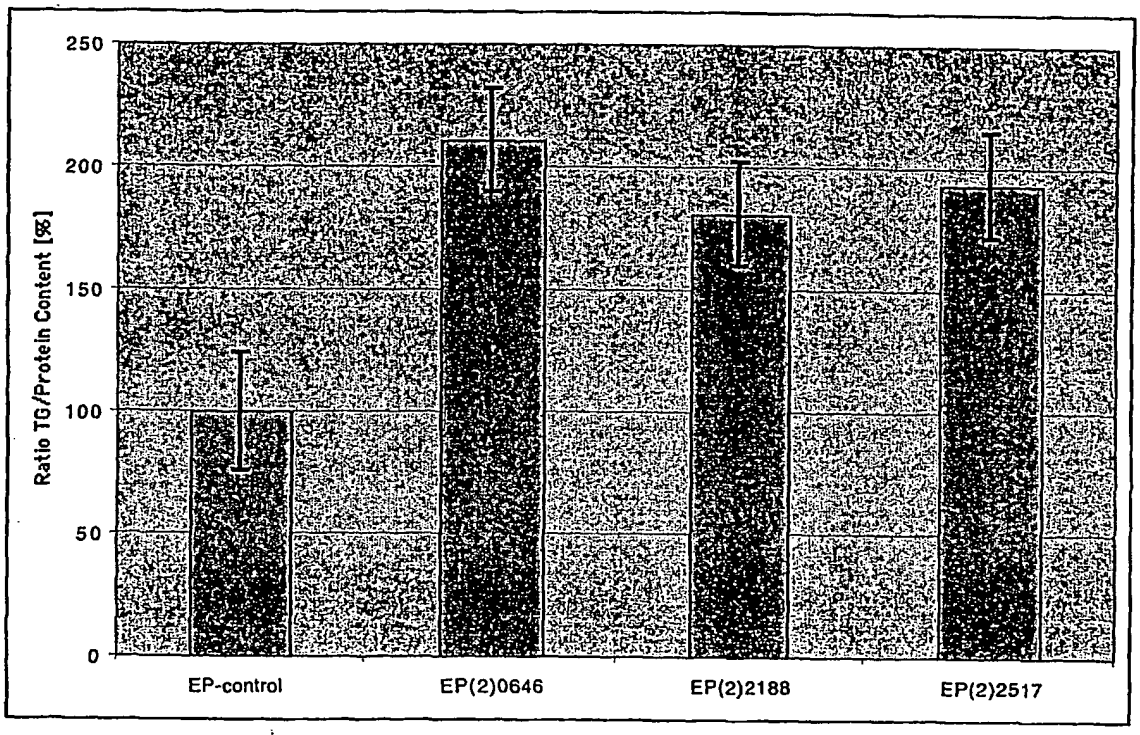


FIGURE 7: Increase of triglyceride content of homozygous flies



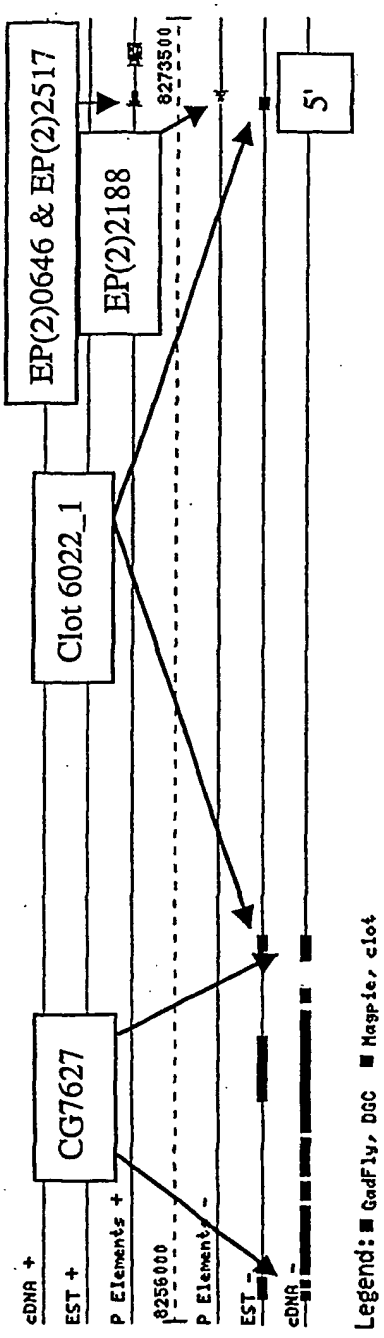


FIGURE 8: Molecular organisation of the *DevG4* locus

FIGURE 9A: Nucleotide sequence of the *DevG4* cDNA (SEQ ID NO:9)

GACCGAAGTT TGTTTTATTA TTTACAATAT CTGTATATAG TTTTTGTGTT GCTTTGCCGG GTCTTCTATC
GCGATTTTCT CACGTTCGCC AGCAACAATA ACCATATTGT TGAATGTAAA CAATATTGAA ACAAAATTTC
TTTAGAGGCA GTAGAACATG CAGTCGCTGA AAACGGCAGA TTTGCCGGAA AATCCCCGGG AACATTGCAA
TTTTATTTCG GCAGCATGTT TTTGGTACAC CATGCCGACT TTTATCAAGG GTCGGAAACG CACATTGGAT
ACCAAGGATC TTTACAGAGC CTTAAAAGAG CACAAATCTG AGACTCTGGG GAACAAATTG TGCGCTTCCT
GGGAATTGGA ACTTGAGAAG ACCAAAGGAA AGCCCAATTT GTTGAGAGCC CTTCTGCGAG TTTTTGGCTG
GTACTTCGCA CTGCTTGGCC TGGTGCTTTT CCTGCTGGAA TTGGGTCTTC GAACGCTTCA GCCAATCTTC
CTGCTGAAAC TCATCGCCTA CTACACACAT GGCTCGGAAT CGATTGAATC GGCCTATTAC TATGCAGCTG
GAGTGATCCT GTGCAGTGCC CTCAATGTCA TTATAATGCA TCCCTATATG CTAGGCACAA TGCATGTGGG
ACTCAAGATG CGCGTTGGTA TGTGCAGCAT GATCTACCGC AAGGCGTTAC GGCTGAGTAA GTCGGCGTTG
GGAGACACCA CAGCTGGACA TGTGGTGAAT CTTATGTCCA ACGACGTGGG ACGTCTCGAT CTGGCTACCA
TATTCGTACA CTACTTGTGG GTGGGACCGC TGGAGACCCT CTTTATCACA TACCTAATGT ACCGTGAGAT
TGGAATCGCT GCTGTGTTTG GTGTGGCCTT CATGTTGCTG TTCATCCCCC TGCAGGCGTA TCTGGGAAAA
AGAACATCGG TGTTGCGACT CAGAACCGCC TTACGCACGG ATGAAAGGGT ACGGATGATG AACGAAATCA
TCTCGGGCAT TCAGGTGATT AAAATGTACG CATGGGAATT GCCATTCGAA CATATGGTGG CCTTTGCCCG
TAAGAAGGAG ATAAATGCCA TCCGCCATGT GTCCTACATC CGTGGAATTC TGCTCTCCTT CATCATCTTT
CTGACGCGTG TCTCAATTTT CCTGAGTCTG GTGGGATATG TTCTGCTCGG GACGTTCCTA ACCCCGGAAG
TGGCGTTCTT GATCACAGCC TACTACAATA TCCTGCGTAC CACTATGACG GTGTTCTTTC CCCAGGGCAT
TTCCCAAATG GCGGAGACCC TGGTGTCCAT TAAGCGTGTC CAGAAGTATA TGCAGTCCGA CGAGACGAAT
GTGATGGATA TGAGTGTGGA TCTTACCGAG GATTTCCAAG GAAGCAATCA GGAAACGGTT CATGCCGATG
GAGATGAGGA GCGCGACGAA GCTGAGGATA AGCTTTTAGG TCCACCAATT GCCACTGTTA ATGAGAACGC
CAAGTTGTCG GAGGCGGGAA TCTCTATTAG CGGACTTATG GCCAAATGGG ATGTTAACTC CCCCGATTAC
TCGCTTAATG GTGTAAACCT TCGTGTTCAG CCTGGAACCA TGCTGGGTAT TGTCGGACGC ACTGGATCCG
GTAAATCCAG TCTCATCCAA GCCATCCTTG GTGAACTGCC CGCAGAGTCT GGCGAGATAA AGGTTAATGG
CTCCATGTCG TATGCTTCCC AAGAACCGTG GCTCTTTTCC GGCACTGTGC GACAAAATAT TCTCTTTGGC
CAGCCTATGG ATCGTCGTCG TTACGCTAAG GTGGTGAAGA AATGTGCCCT GGAGCGAGAT TTCGAGCTGC
TCCCGTTTAA GGATAAAACC ATAGTTGGAG AGCGTGGAGC TTCCCTGTCG GGTGGCCAAA AGGCGAGAAT
CAGTTTGGCA AGAGCTGTTT ATCGGGAGAC CTCCATATAC CTGCTGGATG ATCCTCTGAG TGCCGTGGAC
ACCCATGTGG CCCGCCATCT GTTCGAGCAG TGCATGCGTG GCTATCTACG CGAGCGAATT GTTATATTGG
CCACTCATCA GCTCCAGTTT TTGCAGCACG CCGATCAGAT TGTCATCATG GATAAGGGTC GTGTAAGCGC
CGTGGGCACC TACGAGTCTC TACGCGAATC CGGGTTGGAC TTCGCCTCCA TGCTAGCCGA TCCAGAGCGG
GATGAGCAAT CAGAGGAGCG ATCACGGTCG CGATCGGGCA GCTACACCCA CAGTCATTCG GACCAGCGAC
GCAACAGCGA GCAATCCCTA CTTTCCATGG CAGATTCGTG CATGGATGAC CTCGAAGCGG AGCAAGCTAA
CAACCAGGAA CGCCAGGAGG CTGGTCAAAT CGGCCTGCGC TTGTACAGCA AATACTTCAA AGCGGGAGGC
GGTTTCTTCG CCTTCTTCGT GATGATGGGC TTCTGTGTGC TCTCGCAAGG ATTGGCCTCT CTGGGTGACT
ATTTTCTCTC ATATTGGGTT ACCAAAAAGG GAAATGTGGC TTACCGTGCA GATAATAATG ACACAACTCG
CTCTGAGGAA CTCGAACCTC GTCTGTCGAC ATGGCTTCGT GATATAGGAT TGTCCGTGGA TGCTGAAATG
CTGGATACTT ATATATTCAC GGTGATCACA GTACTGACCA TCCTGGTGAC CGTGGCTCGC TCGTTTTTAT
TCTTTAATTT GGCCATGAAA GCCTCAATTC GTTTGCACAA TTCCATGTTC CGCGGCATCA CCCGAGCTGC
CATGTACTTC TTCAATACGA ATCCATCTGG GCGCATTCTA AACCGTTTCT CAAAGGATAT GGGACAAGTT
GACGAGATAC TGCCTGCCGT GATGATGGAT GTCATCCAGA TTTTCCTTGC ACTTGCTGGC ATTGTGATCG
TCATAGCCGT TGTCAATCCG CTGTTCCTTA TTCCAACCGT AGTACTGGGG ATTATTTTCT ATCAACTGCG
CACCTTTTAT CTAAAGACAT CAAGGGATGT AAAGCGCATG GAAGCAATTA CTCGGTCTCC AGTATACTCG
CATTTAGCTG CCTCGTTGAC CGGTCTGTCC ACCATTCGTG CCTTTGGAGC CCAACGTGTT CTGGAGGCGG
AGTTCGACAA TTACCAGGAT ATGCATAGCT CCGCATTTTA TATGTTCATT AGCACCTCGC GAGCCTTCGG
ATATTGGCTT GACTGTTTCT GTGTGATTTA CATAGCCATA ATTACTCTCA GTTTCTTCAT CTTTCCTCCT
GCGAACGGAG GCGATGTGGG ACTGGCCATT ACGCAGGCAA TGGGAATGAC CGGCATGGTT CAGTGGGGAA
TGCGTCAGTC AGCCGAGCTG GAGAATACGA TGACAGCTGT GGAGCGAGTG GTTGAGTACG AGGACATTGA
ACCGGAAGGA GCGTTGGAAG CTCCGGCCGA TAAGAAGCCA CCAAAGTCAT GGCCAGAGCA GGGAAAAATC
GTTTTCGACG AGCTTAGCTT GCGCTATACG CCGGATCCAA AGTCGGAGAA TGTGCTCAAG TCACTTAGTT
TCGTAATAAA ACCTAAGGAG AAAGTAGGCA TCGTGGGACG CACTGGAGCG GGAAAGTCTT CGCTGATTAA
TGCCCTGTTC CGACTGTCCT ACAACGATGG ATCTGTGCTC ATAGACAAGA GGGATACCAG TGAGATGGGT
TTGCATGACC TGCGCAGCAA AATCTCGATC ATACCGCAGG AACCCGTTCT GTTTTCCGGC ACTATGCGAT
ACAACTTGGA TCCCTTCGAC GAGTATAGCG ATGATAAGCT GTGGCGCTCC CTGGAGGAGG TAAAGCTAAA
GGAGGTGGTT GCTGATCTTC CCAGTGGCTT GCAGAGCAAA ATCACCGAGG GCGGAACCAA CTTCAGCGTT
GGCCAGCGCC AGTTGGTCTG CTTGGCACGG GCTATACTGC GTGAAAATCG TATCCTGGTA ATGGACGAGG
CTACGGCCAA TGTGGATCCC CAGACAGATG GCCTCATCCA AACCACCATA CGAAACAAGT TCAAGGAGTG
CACTGTGCTG ACGATAGCTC ATCGTTTGCA CACCATCATG GACTCGGACA AAGTCTTGGT GATGGACGCT
GGTCGAGCGG TGGAGTTTGG AACGCCCTAT GAACTGCTGA CGCTGGCGGA TTCTAAGGTG TTCCACGGTA
TGGTGAAGCA AACGGGTCAC GCCACCTATG AGAGTCTGCT GAAAATCGCC CAAAAGGCAT TCGAAAACAG
GCAGAATCAC AGTCTTTCCT CGTGAGAATC AGCTATTATG TGTTTGTCAC CGAATCTTTA GCTGGCTAAT
CATAGTTTAA GTAATCATAA TGTTTTTGAA TGTGTTATTT TGTGTGAATG TATATATGTT TTTCCGTGTG
TGTGTGTTAA AAACTTTATA TATGTAACTT AAAAACTGTA TAGGAAACCT TTGTTTAATC TACTATTTGT
ATTTATTAAA CATTCACTAA GAGCAAAGAT GTGCCTTAAA AAATAAATGA AATATTTTCT CTGTTCCTAA

TCTACAA

FIGURE 9B: Amino acid sequence of DevG4 (SEQ ID NO:10)

MQSLKTADLP ENPREHCNFI SAACFWYTMP TFIKGRKRTL DTKDLYRALK EHKSETLGNK LCASWELELE
KTKGKPNLLR ALLRVFGWYF ALLGLVLFLL ELGLRTLQPI FLLKLIAYYT HGSESIESAY YYAAGVILCS
ALNVIIMHPY MLGTMHVGLK MRVGMCSMIY RKALRLSKSA LGDTTAGHVV NLMSNDVGRL DLATIFVHYL
WVGPLETLFI TYLMYREIGI AAVFGVAFML LFIPLQAYLG KRTSVLRLRT ALRTDERVRM MNEIISGIQV
IKMYAWELPF EHMVAFARKK EINAIRHVSY IRGILLSFII FLTRVSIFLS LVGYVLLGTF LTPEVAFLIT
AYYNILRTTM TVFFPQGISQ MAETLVSIKR VQKYMQSDET NVMDMSVDLT EDFQGSNQET VHADGDEERD
EAEDKLLGPP IATVNENAKL SEAGISISGL MAKWDVNSPD YSLNGVNLRV QPGTMLGIVG RTGSGKSSLI
QAILGELPAE SGEIKVNGSM SYASQEPWLF SGTVRQNILF GQPMDRRRYA KVVKKCALER DFELLPFKDK
TIVGERGASL SGGQKARISL ARAVYRETSI YLLDDPLSAV DTHVARHLFE QCMRGYLRER IVILATHQLQ
FLQHADQIVI MDKGRVSAVG TYESLRESGL DFASMLADPE RDEQSEERSR SRSGSYTHSH SDQRRNSEQS
LLSMADSCMD DLEAEQANNQ ERQEAGQIGL RLYSKYFKAG GGFFAFFVMM GFCVLSQGLA SLGDYFLSYW
VTKKGNVAYR ADNNDTTRSE ELEPRLSTWL RDIGLSVDAE MLDTYIFTVI TVLTILVTVA RSFLFFNLAM
KASIRLHNSM FRGITRAAMY FFNTNPSGRI LNRFSKDMGQ VDEILPAVMM DVIQIFLALA GIVIVIAVVN
PLFLIPTVVL GIIFYQLRTF YLKTSRDVKR MEAITRSPVY SHLAASLTGL STIRAFGAQR VLEAEFDNYQ
DMHSSAFYMF ISTSRAFGYW LDCFCVIYIA IITLSFFIFP PANGGDVGLA ITQAMGMTGM VQWGMRQSAE
LENTMTAVER VVEYEDIEPE GALEAPADKK PPKSWPEQGK IVFDELSLRY TPDPKSENVL KSLSFVIKPK
EKVGIVGRTG AGKSSLINAL FRLSYNDGSV LIDKRDTSEM GLHDLRSKIS IIPQEPVLFS GTMRYNLDPF
DEYSDDKLWR SLEEVKLKEV VADLPSGLQS KITEGGTNFS VGQRQLVCLA RAILRENRIL VMDEATANVD
PQTDGLIQTT IRNKFKECTV LTIAHRLHTI MDSDKVLVMD AGRAVEFGTP YELLTLADSK VFHGMVKQTG
HATYESLLKI AQKAFENRQN HSLSS

FIGURE 9C: Nucleotide sequence of the human DevG4 homolog (SEQ ID NO:3)

GGACAGGCGT GGCGGCCGGA GCCCCAGCAT CCCTGCTTGA GGTCCAGGAG CGGAGCCCGC GGCCACCGCC
GCCTGATCAG CGCGACCCCG GCCCGCGCCC GCCCCGCCCG GCAAGATGCT GCCCGTGTAC CAGGAGGTGA
AGCCCAACCC GCTGCAGGAC GCGAACATCT GCTCACGCGT GTTCTTCTGG TGGCTCAATC CCTTGTTTAA
AATTGGCCAT AAACGGAGAT TAGAGGAAGA TGATATGTAT TCAGTGCTGC CAGAAGACCG CTCACAGCAC
CTTGGAGAGG AGTTGCAAGG GTTCTGGGAT AAAGAAGTTT TAAGAGCTGA GAATGACGCA CAGAAGCCTT
CTTTAACAAG AGCAATCATA AAGTGTTACT GGAAATCTTA TTTAGTTTTG GGAATTTTTA CGTTAATTGA
GGAAAGTGCC AAAGTAATCC AGCCCATATT TTTGGGAAAA ATTATTAATT ATTTTGAAAA TTATGATCCC
ATGGATTCTG TGGCTTTGAA CACAGCGTAC GCCTATGCCA CGGTGCTGAC TTTTTGCACG CTCATTTTGG
CTATACTGCA TCACTTATAT TTTTATCACG TTCAGTGTGC TGGGATGAGG TTACGAGTAG CCATGTGCCA
TATGATTTAT CGGAAGGCAC TTCGTCTTAG TAACATGGCC ATGGGGAAGA CAACCACAGG CCAGATAGTC
AATCTGCTGT CCAATGATGT GAACAAGTTT GATCAGGTGA CAGTGTTCTT ACACTTCCTG TGGGCAGGAC
CACTGCAGGC GATCGCAGTG ACTGCCCTAC TCTGGATGGA GATAGGAATA TCGTGCCTTG CTGGGATGGC
AGTTCTAATC ATTCTGCTGC CCTTGCAAAG CTGTTTTGGG AAGTTGTTCT CATCACTGAG GAGTAAAACT
GCAACTTTCA CGGATGCCAG GATCAGGACC ATGAATGAAG TTATAACTGG TATAAGGATA ATAAAAATGT
ACGCCTGGGA AAAGTCATTT TCAAATCTTA TTACCAATTT GAGAAAGAAG GAGATTTCCA AGATTCTGAG
AAGTTCCTGC CTCAGGGGGA TGAATTTGGC TTCGTTTTTC AGTGCAAGCA AAATCATCGT GTTTGTGACC
TTCACCACCT ACGTGCTCCT CGGCAGTGTG ATCACAGCCA GCCGCGTGTT CGTGGCAGTG ACGCTGTATG
GGGCTGTGCG GCTGACGGTT ACCCTCTTCT TCCCCTCAGC CATTGAGAGG GTGTCAGAGG CAATCGTCAG
CATCCGAAGA ATCCAGACCT TTTTGCTACT TGATGAGATA TCACAGCGCA ACCGTCAGCT GCCGTCAGAT
GGTAAAAAGA TGGTGCATGT GCAGGATTTT ACTGCTTTTT GGGATAAGGC ATCAGAGACC CCAACTCTAC
AAGGCCTTTC CTTTACTGTC AGACCTGGCG AATTGTTAGC TGTGGTCGGC CCCGTGGGAG CAGGGAAGTC
ATCACTGTTA AGTGCCGTGC TCGGGGAATT GGCCCCAAGT CACGGGCTGG TCAGCGTGCA TGGAAGAATT
GCCTATGTGT CTCAGCAGCC CTGGGTGTTC TCGGGAACTC TGAGGAGTAA TATTTTATTT GGGAAGAAAT
ATGAAAAGGA ACGATATGAA AAAGTCATAA AGGCTTGTGC TCTGAAAAAG GATTTACAGC TGTTGGAGGA
TGGTGATCTG ACTGTGATAG GAGATCGGGG AACCACGCTG AGTGGAGGGC AGAAAGCACG GGTAAACCTT
GCAAGAGCAG TGTATCAAGA TGCTGACATC TATCTCCTGG ACGATCCTCT CAGTGCAGTA GATGCGGAAG
TTAGCAGACA CTTGTTCGAA CTGTGTATTT GTCAAATTTT GCATGAGAAG ATCACAATTT TAGTGACTCA
TCAGTTGCAG TACCTCAAAG CTGCAAGTCA GATTCTGATA TTGAAAGATG GTAAAATGGT GCAGAAGGGG
ACTTACACTG AGTTCCTAAA ATCTGGTATA GATTTTGGCT CCCTTTTAAA GAAGGATAAT GAGGAAAGTG
AACAACCTCC AGTTCCAGGA ACTCCCACAC TAAGGAATCG TACCTTCTCA GAGTCTTCGG TTTGGTCTCA
ACAATCTTCT AGACCCTCCT TGAAAGATGG TGCTCTGGAG AGCCAAGATA CAGAGAATGT CCCAGTTACA
CTATCAGAGG AGAACCGTTC TGAAGGAAAA GTTGGTTTTC AGGCCTATAA GAATTACTTC AGAGCTGGTG
CTCACTGGAT TGTCTTCATT TTCCTTATTC TCCTAAACAC TGCAGCTCAG GTTGCCTATG TGCTTCAAGA
TTGGTGGCTT TCATACTGGG CAAACAAACA AAGTATGCTA AATGTCACTG TAAATGGAGG AGGAAATGTA
ACCGAGAAGC TAGATCTTAA CTGGTACTTA GGAATTTATT CAGGTTTAAC TGTAGCTACC GTTCTTTTTG

GCATAGCAAG ATCTCTATTG GTATTCTACG TCCTTGTTAA CTCTTCACAA ACTTTGCACA ACAAAATGTT
TGAGTCAATT CTGAAAGCTC CGGTATTATT CTTTGATAGA AATCCAATAG GAAGAATTTT AAATCGTTTC
TCCAAAGACA TTGGACACTT GGATGATTTG CTGCCGCTGA CGTTTTTAGA TTTCATCCAG ACATTGCTAC
AAGTGGTTGG TGTGGTCTCT GTGGCTGTGG CCGTGATTCC TTGGATCGCA ATACCCTTGG TTCCCCTTGG
AATCATTTTC ATTTTTCTTC GGCGATATTT TTTGGAAACG TCAAGAGATG TGAAGCGCCT GGAATCTACA
ACTCGGAGTC CAGTGTTTTC CCACTTGTCA TCTTCTCTCC AGGGGCTCTG GACCATCCGG GCATACAAAG
CAGAAGAGAG GTGTCAGGAA CTGTTTGATG CACACCAGGA TTTACATTCA GAGGCTTGGT TCTTGTTTTT
GACAACGTCC CGCTGGTTCG CCGTCCGTCT GGATGCCATC TGTGCCATGT TTGTCATCAT CGTTGCCTTT
GGGTCCCTGA TTCTGGCAAA AACTCTGGAT GCCGGGCAGG TTGGTTTGGC ACTGTCCTAT GCCCTCACGC
TCATGGGGAT GTTTCAGTGG TGTGTTCGAC AAAGTGCTGA AGTTGAGAAT ATGATGATCT CAGTAGAAAG
GGTCATTGAA TACACAGACC TTGAAAAAGA AGCACCTTGG GAATATCAGA AACGCCCACC ACCAGCCTGG
CCCCATGAAG GAGTGATAAT CTTTGACAAT GTGAACTTCA TGTACAGTCC AGGTGGGCCT CTGGTACTGA
AGCATCTGAC AGCACTCATT AAATCACAAG AAAAGGTTGG CATTGTGGGA AGAACCGGAG CTGGAAAAAG
TTCCCTCATC TCAGCCCTTT TTAGATTGTC AGAACCCGAA GGTAAAATTT GGATTGATAA GATCTTGACA
ACTGAAATTG GACTTCACGA TTTAAGGAAG AAAATGTCAA TCATACCTCA GGAACCTGTT TTGTTCACTG
GAACAATGAG GAAAAACCTG GATCCCTTTA AGGAGCACAC GGATGAGGAA CTGTGGAATG CCTTACAAGA
GGTACAACTT AAAGAAACCA TTGAAGATCT TCCTGGTAAA ATGGATACTG AATTAGCAGA ATCAGGATCC
AATTTTAGTG TTGGACAAAG ACAACTGGTG TGCCTTGCCA GGGCAATTCT CAGGAAAAAT CAGATATTGA
TTATTGATGA AGCGACGGCA AATGTGGATC CAAGAACTGA TGAGTTAATA CAAAAAAAAA TCCGGGAGAA
ATTTGCCCAC TGCACCGTGC TAACCATTGC ACACAGATTG AACACCATTA TTGACAGCGA CAAGATAATG
GTTTTAGATT CAGGAAGACT GAAAGAATAT GATGAGCCGT ATGTTTTGCT GCAAAATAAA GAGAGCCTAT
TTTACAAGAT GGTGCAACAA CTGGGCAAGG CAGAAGCCGC TGCCCTCACT GAAACAGCAA AACAGGTATA
CTTCAAAAGA AATTATCCAC ATATTGGTCA CACTGACCAC ATGGTTACAA ACACTTCCAA TGGACAGCCC
TCGACCTTAA CTATTTTCGA GACAGCACTG TGAATCCAAC CAAAATGTCA AGTCCGTTCC GAAGGCATTT
TCCACTAGTT TTTGGACTAT GTAAACCACA TTGTACTTTT TTTTACTTTG GCAACAAATA TTTATACATA
CAAGATGCTA GTTCATTTGA ATATTTCTCC C

FIGURE 9D: Amino acid sequence of the human DevG4 homolog (SEQ ID NO:4)

MLPVYQEVKP NPLQDANICS RVFFWWLNPL FKIGHKRRLE EDDMYSVLPE DRSQHLGEEL QGFWDKEVLR
AENDAQKPSL TRAIIKCYWK SYLVLGIFTL IEESAKVIQP IFLGKIINYF ENYDPMDSVA LNTAYAYATV
LTFCTLILAI LHHLYFYHVQ CAGMRLRVAM CHMIYRKALR LSNMAMGKTT TGQIVNLLSN DVNKFDQVTV
FLHFLWAGPL QAIAVTALLW MEIGISCLAG MAVLIILLPL QSCFGKLFSS LRSKTATFTD ARIRTMNEVI
TGIRIIKMYA WEKSFSNLIT NLRKKEISKI LRSSCLRGMN LASFFSASKI IVFVTFTTYV LLGSVITASR
VFVAVTLYGA VRLTVTLFFP SAIERVSEAI VSIRRIQTFL LLDEISQRNR QLPSDGKKMV HVQDFTAFWD
KASETPTLQG LSFTVRPGEL LAVVGPVGAG KSSLLSAVLG ELAPSHGLVS VHGRIAYVSQ QPWVFSGTLR
SNILFGKKYE KERYEKVIKA CALKKDLQLL EDGDLTVIGD RGTTLSGGQK ARVNLARAVY QDADIYLLDD
PLSAVDAEVS RHLFELCICQ ILHEKITILV THQLQYLKAA SQILILKDGK MVQKGTYTEF LKSGIDFGSL
LKKDNEESEQ PPVPGTPTLR NRTFSESSVW SQQSSRPSLK DGALESQDTE NVPVTLSEEN RSEGKVGFQA
YKNYFRAGAH WIVFIFLILL NTAAQVAYVL QDWWLSYWAN KQSMLNVTVN GGGNVTEKLD LNWYLGIYSG
LTVATVLFGI ARSLLVFYVL VNSSQTLHNK MFESILKAPV LFFDRNPIGR ILNRFSKDIG HLDDLLPLTF
LDFIQTLLQV VGVVSVAVAV IPWIAIPLVP LGIIFIFLRR YFLETSRDVK RLESTTRSPV FSHLSSSLQG
LWTIRAYKAE ERCQELFDAH QDLHSEAWFL FLTTSRWFAV RLDAICAMFV IIVAFGSLIL AKTLDAGQVG
LALSYALTLM GMFQWCVRQS AEVENMMISV ERVIEYTDLE KEAPWEYQKR PPPAWPHEGV IIFDNVNFMY
SPGGPLVLKH LTALIKSQEK VGIVGRTGAG KSSLISALFR LSEPEGKIWI DKILTTEIGL HDLRKKMSII
PQEPVLFTGT MRKNLDPFKE HTDEELWNAL QEVQLKETIE DLPGKMDTEL AESGSNFSVG QRQLVCLARA
ILRKNQILII DEATANVDPR TDELIQKKIR EKFAHCTVLT IAHRLNTIID SDKIMVLDSG RLKEYDEPYV
LLQNKESLFY KMVQQLGKAE AAALTETAKQ VYFKRNYPHI GHTDHMVTNT SNGQPSTLTI FETAL

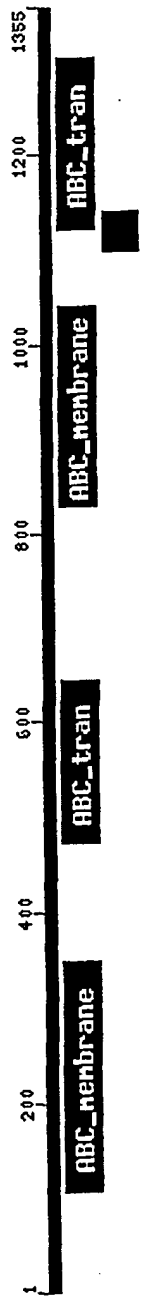


FIGURE 10: Protein domains of the DevG4 protein

FIGURE 11: Comparison of human MRP4, mouse MRP4 (partial), and Drosophila DevG4 protein domains

(A) CLUSTAL X (1.8) multiple sequence alignment of ABC-membrane I

```
hMRP4          YLVLG--IFTLIEESAKVIQPIFLGKIINYFENYDPMDSVALNTAYAYATVLTFCTLILA
DevG4          YFALLGLVLFLLELGLRTLQPIFLLKLIAYYT----HGSESIESAYYYAAGVILCSALNV

hMRP4          ILHHLYFYHVQCAGMRLRVAMCHMIYRKALRLSNMAMGKTTTGQIVNLLSNDVNKFDQVT
DevG4          IIMHPYMLGTMHVGLKMRVGMCSMIYRKALRLSKSALGDTTAGHVVNLMSNDVGRLDLAT

hMRP4          VFLHFLWAGPLQAIAVTALLWMEIGISCLAGMAVLIILLPLQSCFGKLFSSLRSKTATFT
DevG4          IFVHYLWVGPLETLFITYLMYREIGIAAVFGVAFMLLFIPLQAYLGKRTSVLRLRTALRT

hMRP4          DARIRTMNEVITGIRIIKMYAWEKSFSNLITNLRKKEISKILRSSCLRGMNLASFFSASK
DevG4          DERVRMMNEIISGIQVIKMYAWELPFEHMVAFARKKEINAIRHVSYIRGILLSFIIFLTR

hMRP4          IIVFVTFTTYVLLGSVITASRVFVAVTLYGAVRLTV
DevG4          VSIFLSLVGYVLLGTFLTPEVAFLITAYYNILRTTM
```

(B) CLUSTAL X (1.8) multiple sequence alignment of ABC-tran I

```
hMRP4          GELLAVVGPVGAGKSSLLSAVLGELAPSHGLVSVHGRIAYVSQQPWVFSGTLRSNILFGK
DevG4          GTMLGIVGRTGSGKSSLIQAILGELPAESGEIKVNGSMSYASQEPWLFSGTVRQNILFGQ

hMRP4          KYEKERYEKVIKACALKKDLQLLEDGDLTVIGDRGTTLSGGQKARVNLARAVYQDADIYL
DevG4          PMDRRRYAKVVKKCALERDFELLPFKDKTIVGERGASLSGGQKARISLARAVYRETSIYL

hMRP4          LDDPLSAVDAEVSRHLFELCICQILHEKITILVTHQLQYLKAASQILILKDG
DevG4          LDDPLSAVDTHVARHLFEQCMRGYLRERIVILATHQLQFLQHADQIVIMDKG
```

(C) CLUSTAL X (1.8) multiple sequence alignment of ABC- membrane II

```
hMRP4          WIVFIFLILLNTAAQVAYVLQDWWLSYWANKQ-SMLNVTVNGGGNVTEKLD--LNWYLG-
DevG4          FVMMGFCVLSQGLASLG----DYFLSYWVTKKGNVAYRADNNDTTRSEELEPRLSTWLRD

hMRP4          --------------IYSGLTVATVLFGIARSLLVFYVLVNSSQTLHNKMFESILKAPVLFF
DevG4          IGLSVDAEMLDTYIFTVITVLTILVTVARSFLFFNLAMKASIRLHNSMFRGITRAAMYFF

hMRP4          DRNPIGRILNRFSKDIGHLDDLLPLTFLDFIQTLLQVVGVVSVAVAVIPWIAIPLVPLGI
DevG4          NTNPSGRILNRFSKDMGQVDEILPAVMMDVIQIFLALAGIVIVIAVVNPLFLIPTVVLGI

hMRP4          IFIFLRRYFLETSRDVKRLESTTRSPVFSHLSSSLQGLWTIRAYKAEERCQELFDAHQDL
DevG4          IFYQLRTFYLKTSRDVKRMEAITRSPVYSHLAASLTGLSTIRAFGAQRVLEAEFDNYQDM

hMRP4          HSEAWFLFLTTSRWFAVRLDAICAMFVIIVAFGSLILAKTLDAGQVGLALSYALTLMGMF
DevG4          HSSAFYMFISTSRAFGYWLDCFCVIYIAIITLSFFIFP-PANGGDVGLAITQAMGMTGMV

hMRP4          QWCV
DevG4          QWGM
```

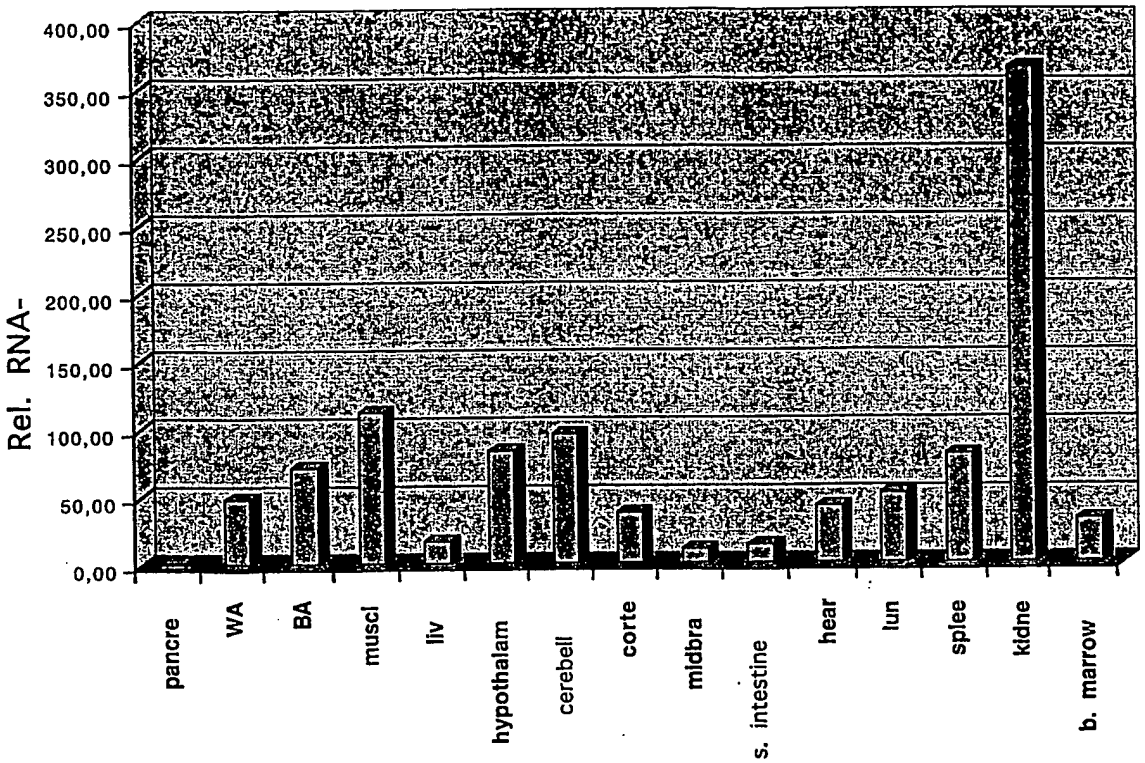
(D) CLUSTAL X (1.8) multiple sequence alignment of ABC-tran II

```
hMRP4          -EKVGIVGRTGAGKSSLISALFRLSEPEGKIWIDKILTTEIGLHDLRKKMSIIPQEPVLF
mMRP4          REKVGIVGRTGAGKSSLISALFRLSEPEGKIWIDKILTTEIGLHDLRKKMSIIPQEPVLF
DevG4          KEKVGIVGRTGAGKSSLINALFRLSYNDGSVLIDKRDTSEMGLHDLRSKISIIPQEPVLF

hMRP4          TGTMRKNLDPFKEHTDEELWNALQEVQLKETIEDLPGKMDTELAESGSNFSVGQRQLVCL
mMRP4          TGTMRKNLDPFNEHTDEELWRALEEVQLKEAIEDLPGKMDTELAESGSNFSVGQRQLVCL
```

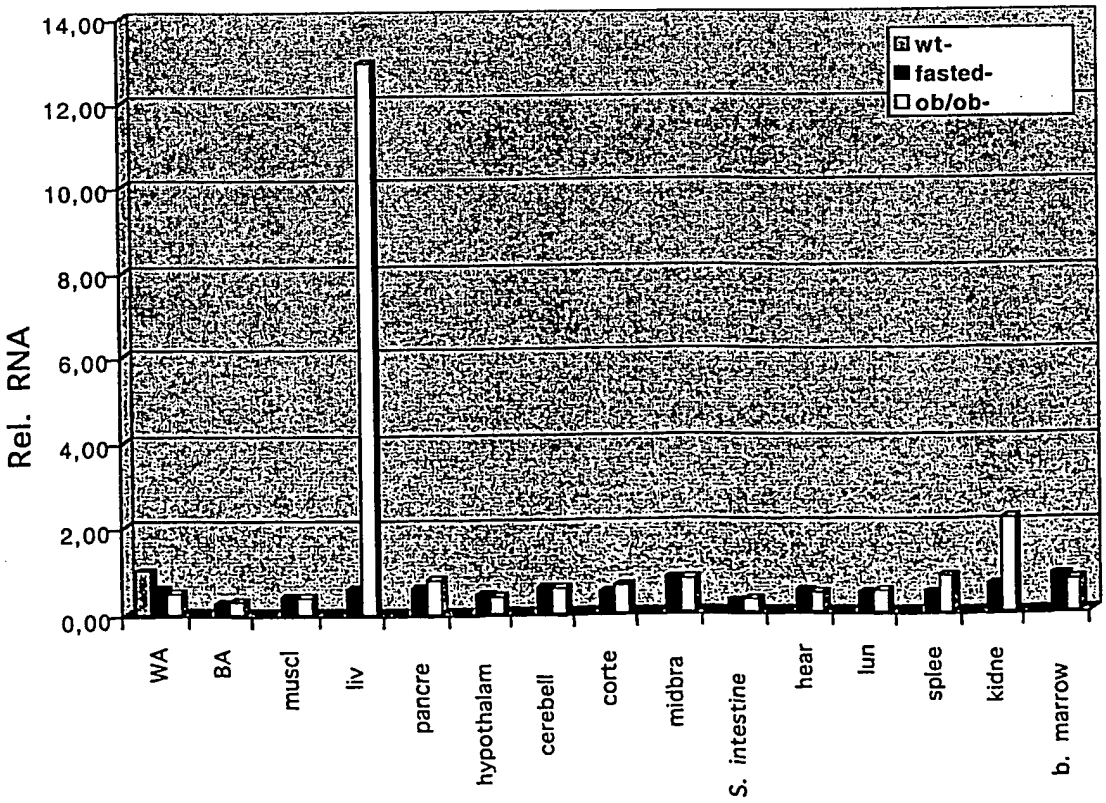
```
DevG4           SGTMRYNLDPFDEYSDDKLWRSLEEVKLKEVVADLPSGLQSKITEGGTNFSVGQRQLVCL

hMRP4           ARAILRKNQILIIDEATANVDPRTDELIQKKIREKFAHCTVLTIAHRLNTIIDSDKIMVL
mMRP4           A------------------------------------------------------------
DevG4           ARAILRENRILVMDEATANVDPQTDGLIQTTIRNKFKECTVLTIAHRLHTIMDSDKVLVM

hMRP4           DSG
mMRP4           ---
DevG4           DAG
```

FIGURE 12: Expression of DevG4 (MRP4) in mammalian tissues

(A) Real-time PCR analysis of DevG4 (MRP4) expression in wildtype mouse tissues



(B) Real-time PCR mediated comparison of DevG4 (MRP4) expression in different mouse models



(C) Real-time PCR mediated comparison of DevG4 (MRP4) expression during the differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes

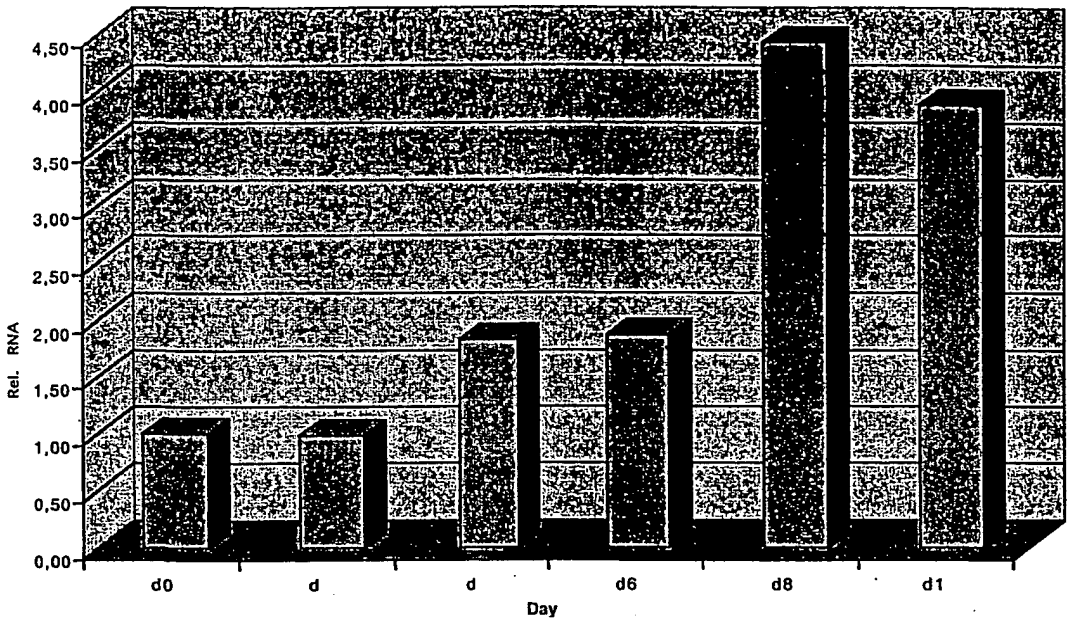
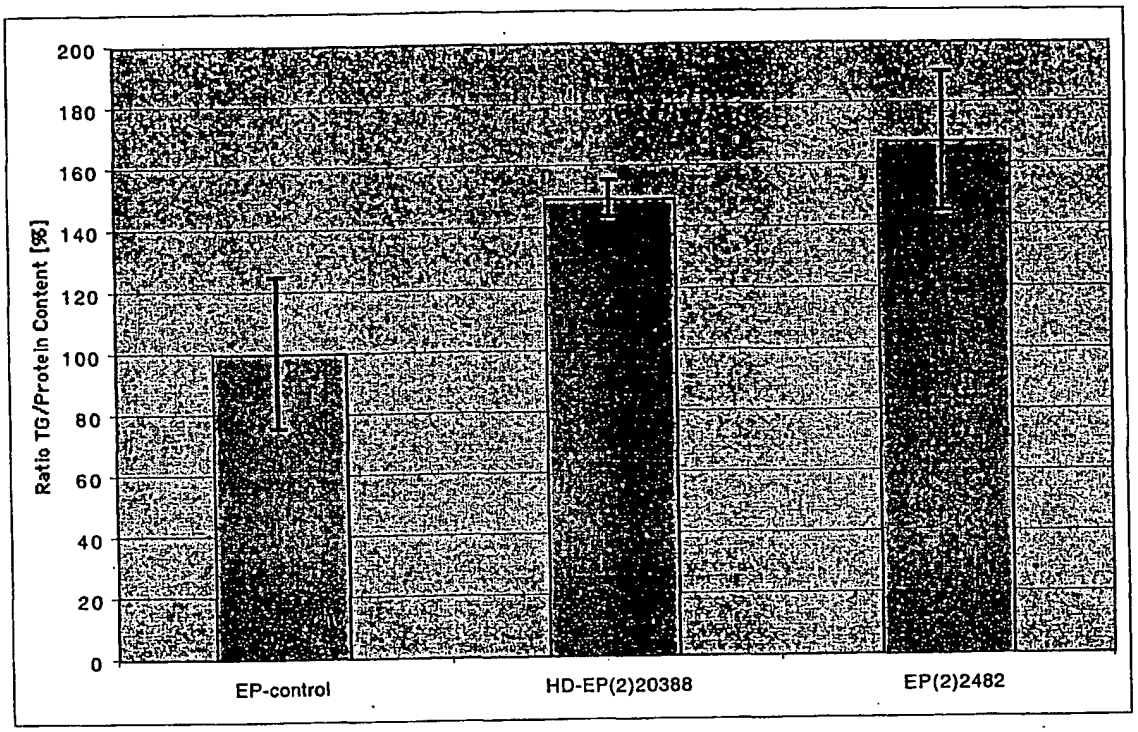


FIGURE 13: Increase of triglyceride content of homozygous flies



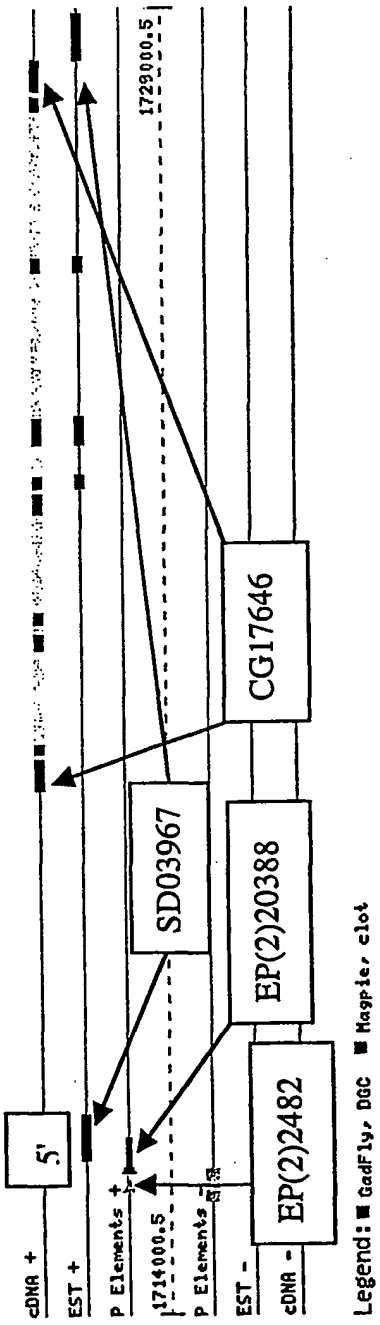


FIGURE 14: Molecular organisation of the *DevG22* locus

FIGURE 15A: Nucleotide sequence of the *DevG22* cDNA (SEQ ID NO:11)

```
CGTGGCGAGC GCACGTGCAG CGGAACGTAT ATATTCTTGT TCAGCGGGAT CGGAATCGGG GAAAATCGAG
ACATATACAG AAATCGAAGC ACATTATGTA TAACACACGC AATTGAAATT AATTTACGCG TCGCTGCCCA
TCTGCATAAG TTCAATGGAA GCAGAGGCAA AAGTTTGAGC AAAAGCACAG ACCGCGTACC GAGAGGAAAA
GTAGAAAAAT GTGAAGAAAG TGCAACAAAG TCGAATTAGT AAATCAACCA AAAAAAAAAA ATACAAGAAG
TGCACCTAAC AACAATAAAA TAAAACAATC ATCGCGTGTT CGTCCATATG TTAAACAAAG GTTAATTAAA
CAGACTGCAA TTATTCCGCG TTGCAGTTGG CAGTTGGTGG AGTACAAGAA ACATTTCTTT GTGTTTATGT
GTTTTCATTC AGAAATATTC GTTACGCGTC TACTCTTACG ACTTCCCGAG AAGAATCCGA ATAATTTGTG
TACTACTGCG AAGAAAGAGA CGCTGAAGAG CTTGCAGTCA AGAGATACCC CTTCTGACTA GAAACATTGA
GGATTCCGTG CAAACATGAC CCTCTTGCCA GACATCAAGG CATCGGATGC CGCCTGCTGC GTGGGAGGTG
CGAACAGCGA TGCCAGCAGC AGTTCCGGCG TCTGCAGCCT CAAGGGTCAC CATGGTGACC CCGACAGCGG
TTGCCTGGGC ATCAACTGTT GCACCACGGC GGCCAGCTCG GAGCTGTCCA TCGACGAGAC GTCATCGACC
TCGTCCAGAG GCTCCTGCGA GTTGACCAGC AAGGTGACCA ACGACCTGAA CGGATTCCAG AGTCCGAACT
ACCACCAGGC GGTGGCGCAC GCCAACTTCG ACCACTGCGA TCCCGTGGAC ATCCAGTTCG CCGACGTGCG
CTACACAGTG AAGAAGTTCT CCTTCCCGGA GCGGAAGTTC GTCACCAAGG AGATCCTTCA TGGCCTGAAC
GGCAGCTTCC GGTCTGGCGA ACTCACAGCC ATCATGGGTC CTTCGGGCGC CGGCAAGAGC ACGCTGCTGA
ATGTGATGTC CGGATTTTGT AATGGACGTG GTGGTAAAGA CGATGCCCCA GGCGGTACAC GGGTTTTCAT
TGCTAAACGT TATCGCTCCA CTGGCGTGTC GGGCGATATC CGGGTTAATA GAAAGCCCAT GGCTCCCAGC
TCCGAGAGGT TCCGCCAAAT GCTGTGCTAC ATCCACCAGG ACGATCTGCT GCGTCCACAG CTGTTGGTCG
GCGAGATAAT GCTGCTGGCG GCACATCTGA AGCTGGGCTT CAAGGTCACC AAGGCGTACA AGATGGATCT
GATCAAGCAC ATCTTATCGC TGCTGGGTCT GGACCATCGC TACAATGTGC CCACTGGGAA GCTTTCGGGT
GGCCAGAAGA AGCGACTCGC AATCGCCCTG GAGCTGATAA GTAATCCTCC CGTGCTATAT CTGGATGAGC
CGACGACTGG CCTGGACAGC TCCTCGTGCA GCTCCTGCGT GGCTCTGCTG AAGAAACTGG CGTCGCAGGG
CCACACGATA GTCTGCACCA TCCATCAGCC AAGTGCCCTC ATCTTCGAGA TGTTCGACAA GCTCTACACC
GTCGTCGATG GCCACTGCAT GTACCAAGGA CCTGTGCGCG AACTGGTGCC CTTCCTGGCC GACCAGCAGC
TCGTCTGCCC GAGTTACCAC AACCCAGCTG ACTATCTACT GGAAGTGGCC GTGGGCGAGC ATCAACGTGA
CCTGAATGAG CTAATCCATG CGGCCAATAA AAAGTATTAC GAGGATGTGG ATCGCCATAG GTATATGAGC
AGTGATGATA TGGCACGCCT CGTGGAAAGC ATTAAAGAAA ACATGGGCGG CAAGGCAGTG GTAAAAACCA
GTGAAGCGCT GGCAGCATTT GCGGCGGCGC AATTCTCCAG CTTCGACTAT GTAAAGCCCT CGCCGCAGGA
GCTGGCTCTG GAGGAGATCA AGGCACTGAG CGGCGGCCCC GAGAGCGCGG ATCCCGATCT CCTCGAGAAA
AATCTGAGGC CACAGCCACA GCCGCTTGCC AAAGCCGGTG AGCTTGCCAG GCCGCCGAAT GCCATTCGAT
CGGCCTCGTT CCTCATGCAG TATGTGCTCC TGATGCAGCG CATCTTGATT TGCGCCAAGC GAAACTACTT
TCTGCTGCTG GCCCGCATCT TCTCGCACAT TTTCATCGGA GTCGTCTTCG GGTATCTGTA CATGAACGTG
GGCAACAATG CCCAGAGTGT GCTGGGAAAC TACGTGTATC TGTACGGCTC CACGCTGCTC TTGGTCTACA
CCGGTAAAAT GGCTGTGGTC TTGACATTTC CGCTGGAAAT TGACATGTTG ACACGGGAGC ACTTCAACCG
CTGGTACAAA CTGGGTCCCT ACTTCCTCTC GTTGATCTCC TTCGAAATAC CCTTCCAGGT GAGCACCGCC
ATAGAATAG
```

FIGURE 15B: Amino acid sequence of DevG22 (SEQ ID NO:12)

```
MTLLPDIKAS DAACCVGGAN SDASSSSGVC SLKGHHGDPD SGCLGINCCT TAASSELSID ETSSTSSRGS
CELTSKVTND LNGFQSPNYH QAVAHANFDH CDPVDIQFAD VRYTVKKFSF PERKFVTKEI LHGLNGSFRS
GELTAIMGPS GAGKSTLLNV MSGFCNGRGG KDDAPGGTRV FIAKRYRSTG VSGDIRVNRK PMAPSSERFR
QMLCYIHQDD LLRPQLLVGE IMLLAAHLKL GFKVTKAYKM DLIKHILSLL GLDHRYNVPT GKLSGGQKKR
LAIALELISN PPVLYLDEPT TGLDSSSCSS CVALLKKLAS QGHTIVCTIH QPSALIFEMF DKLYTVVDGH
CMYQGPVREL VPFLADQQLV CPSYHNPADY LLEVAVGEHQ RDLNELIHAA NKKYYEDVDR HRYMSSDDMA
RLVESIKENM GGKAVVKTSE ALAAFAAAQF SSFDYVKPSP QELALEEIKA LSGGPESADP DLLEKNLRPQ
PQPLAKAGEL ARPPNAIRSA SFLMQYVLLM QRILICAKRN YFLLLARIFS HIFIGVVFGY LYMNVGNNAQ
SVLGNYVYLY GSTLLLVYTG KMAVVLTFPL EIDMLTREHF NRWYKLGPYF LSLISFEIPF QVSTAIE
```

FIGURE 15C: Nucleotide sequence of the human DevG22 homolog (SEQ ID NO:5)

```
GCTTTATAAA GGGGAGTTTC CCTGCACAAG CTCTCTCTCT TGTCTGCCGC CATGTGAGAC ATGCCTTTCA
CCTTCCGCCA TGATCATGAG GCTTCCCCAG CCACATGGAA CTAATGCCAG CAGTTACTCT GCAGAGATGA
CGGAGCCCAA GTCGGTGTGT GTCTCGGTGG ATGAGGTGGT GTCCAGCAAC ATGGAGGCCA CTGAGACGGA
CCTGCTGAAT GGACATCTGA AAAAAGTAGA TAATAACCTC ACGGAAGCCC AGCGCTTCTC CTCCTTGCCT
CGGAGGGCAG CTGTGAACAT TGAATTCAGG GACCTTTCCT ATTCGGTTCC TGAAGGACCC TGGTGGAGGA
AGAAAGGATA CAAGACCCTC CTGAAAGGAA TTTCCGGGAA GTTCAATAGT GGTGAGTTGG TGGCCATTAT
GGGTCCTTCC GGGGCCGGGA AGTCCACGCT GATGAACATC CTGGCTGGAT ACAGGGAGAC GGGCATGAAG
GGGGCCGTCC TCATCAACGG CCTGCCCCGG GACCTGCGCT GCTTCCGGAA GGTGTCCTGC TACATCATGC
AGGATGACAT GCTGCTGCCG CATCTCACTG TGCAGGAGGC CATGATGGTG TCGGCACATC TGAAGCTTCA
```

GGAGAAGGAT GAAGGCAGAA GGGAAATGGT CAAGGAGATA CTGACAGCGC TGGGCTTGCT GTCTTGCGCC
AACACGCGGA CCGGGAGCCT GTCAGGTGGT CAGCGCAAGC GCCTGGCCAT CGCGCTGGAG CTGGTGAACA
ACCCTCCAGT CATGTTCTTC GATGAGCCCA CCAGCGGCCT GGACAGCGCC TCCTGCTTCC AGGTGGTCTC
GCTGATGAAA GGGCTCGCTC AAGGGGGTCG CTCCATCATT TGCACCATCC ACCAGCCCAG CGCCAAACTC
TTCGAGCTGT TCGACCAGCT TTACGTCCTG AGTCAAGGAC AATGTGTGTA CCGGGGAAAA GTCTGCAATC
TTGTGCCATA TTTGAGGGAT TTGGGTCTGA ACTGCCCAAC CTACCACAAC CCAGCAGATT TTGTCATGGA
GGTTGCATCC GGCGAGTACG GTGATCAGAA CAGTCGGCTG GTGAGAGCGG TTCGGGAGGG CATGTGTGAC
TCAGACCACA AGAGAGACCT CGGGGGTGAT GCCGAGGTGA ACCCTTTTCT TTGGCACCGG CCCTCTGAAG
AGGACTCCTC GTCCATGGAA GGCTGCCACA GCTTCTCTGC CAGCTGCCTC ACGCAGTTCT GCATCCTCTT
CAAGAGGACC TTCCTCAGCA TCATGAGGGA CTCGGTCCTG ACACACCTGC GCATCACCTC GCACATTGGG
ATCGGCCTCC TCATTGGCCT GCTGTACTTG GGGATCGGGA ACGAAGCCAA GAAGGTCTTG AGCAACTCCG
GCTTCCTCTT CTTCTCCATG CTGTTCCTCA TGTTCGCGGC CCTCATGCCT ACTGTTCTGA CATTTCCCCT
GGAGATGGGA GTCTTTCTTC GGGAACACCT GAACTACTGG TACAGCCTGA AGGCCTACTA CCTGGCCAAG
ACCATGGCAG ACGTGCCCTT TCAGATCATG TTCCCAGTGG CCTACTGCAG CATCGTGTAC TGGATGACGT
CGCAGCCGTC CGACGCCGTG CGCTTTGTGC TGTTTGCCGC GCTGGGCACC ATGACCTCCC TGGTGGCACA
GTCCCTGGGC CTGCTGATCG GAGCCGCCTC CACGTCCCTG CAGGTGGCCA CTTTCGTGGG CCCAGTGACA
GCCATCCCGG TGCTCCTGTT CTCGGGGTTC TTCGTCAGCT TCGACACCAT CCCCACGTAC CTACAGTGGA
TGTCCTACAT CTCCTATGTC AGGTATGGGT TCGAAGGGGT CATCCTCTCC ATCTATGGCT TAGACCGGGA
AGATCTGCAC TGTGACATCG ACGAGACGTG CCACTTCCAG AAGTCGGAGG CCATCCTGCG GGAGCTGGAC
GTGGAAAATG CCAAGCTGTA CCTGGACTTC ATCGTACTCG GGATTTTCTT CATCTCCCTC CGCCTCATTG
CCTATTTTGT CCTCAGGTAC AAAATCCGGG CAGAGAGGTA AAACACCTGA ATGCCAGGAA ACAGGAAGAT
TAGACACTGT GGCCGAGGGC ACGTCTAGAA TCGAGGAGGC AAGCCTGTGC CCGACCGACG ACACAGAGAC
TCTTCTGATC CAACCCCTAG AACCGCGTTG GGTTTGTGGG TGTCTCGTGC TCAGCCACTC TGCCCAGCTG
GGTTGGATCT TCTCTCCATT CCCCTTTCTA GCTTTAACTA GGAAGATGTA GGCAGATTGG TGGTTTTTTT
TTTTTTAACA TACAGAATTT TAAATACCAC AACTGGGGCA GAATTTAAAG CTGCAACACA GCTGGTGATG
AGAGGCTTCC TCAGTCCAGT CGCTCCTTAG CACCAGGCAC CGTGGGTCCT GGATGGGGAA CTGCAAGCAG
CCTCTCAGCT GATGGCTGCA CAGTCAGATG TCTGGTGGCA GAGAGTCCGA GCATGGAGCG ATTCCATTTT
ATGACTGTTG TTTTTCACAT TTTCATCTTT CTAAGGTGTG TCTCTTTTCC AATGAGAAGT CATTTTTGCA
AGCCAAAAGT CGATCAATCG CATTCATTTT AAGAAATTAT ACCTTTTTAG TACTTGCTGA AGAATGATTC
AGGGTAAATC ACATACTTTG TTTAGAGAGG CGAGGGGTTT AACCGAGTCA CCCAGCTGGT CTCATACATA
GACAGCACTT GTGAAGGATT GAATGCAGGT TCCAGGTGGA GGGAAGACGT GGACACCATC TCCACTGAGC
CATGCAGACA TTTTTAAAAG CTATACAAAA AATTGTGAGA AGACATTGGC CAACTCTTTC AAAGTCTTTC
TTTTTCCACG TGCTTCTTAT TTTAAGCGAA ATATATTGTT TGTTTCTTCC T

FIGURE 15D: Amino acid sequence of the human DevG22 homolog (SEQ ID NO:6)

MIMRLPQPHG TNASSYSAEM TEPKSVCVSV DEVVSSNMEA TETDLLNGHL KKVDNNLTEA QRFSSLPRRA
AVNIEFRDLS YSVPEGPWWR KKGYKTLLKG ISGKFNSGEL VAIMGPSGAG KSTLMNILAG YRETGMKGAV
LINGLPRDLR CFRKVSCYIM QDDMLLPHLT VQEAMMVSAH LKLQEKDEGR REMVKEILTA LGLLSCANTR
TGSLSGGQRK RLAIALELVN NPPVMFFDEP TSGLDSASCF QVVSLMKGLA QGGRSIICTI HQPSAKLFEL
FDQLYVLSQG QCVYRGKVCN LVPYLRDLGL NCPTYHNPAD FVMEVASGEY GDQNSRLVRA VREGMCDSDH
KRDLGGDAEV NPFLWHRPSE EDSSSMEGCH SFSASCLTQF CILFKRTFLS IMRDSVLTHL RITSHIGIGL
LIGLLYLGIG NEAKKVLSNS GFLFFSMLFL MFAALMPTVL TFPLEMGVFL REHLNYWYSL KAYYLAKTMA
DVPFQIMFPV AYCSIVYWMT SQPSDAVRFV LFAALGTMTS LVAQSLGLLI GAASTSLQVA TFVGPVTAIP
VLLFSGFFVS FDTIPTYLQW MSYISYVRYG FEGVILSIYG LDREDLHCDI DETCHFQKSE AILRELDVEN
AKLYLDFIVL GIFFISLRLI AYFVLRYKIR AER

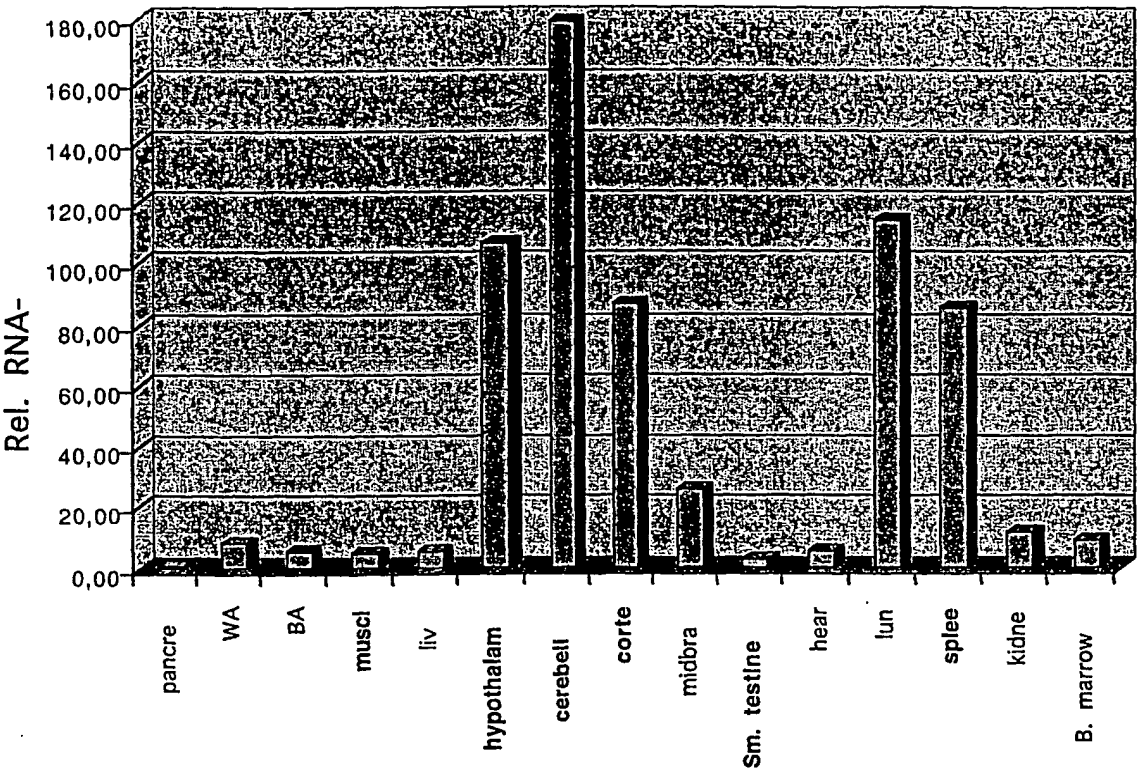
FIGURE 16: Protein domains of the DevG22 protein

FIGURE 17: CLUSTAL X (1.8) multiple sequence alignment of DevG22 proteins from human (hDevG22), mouse (mDevG22), and Drosophila (DevG22).

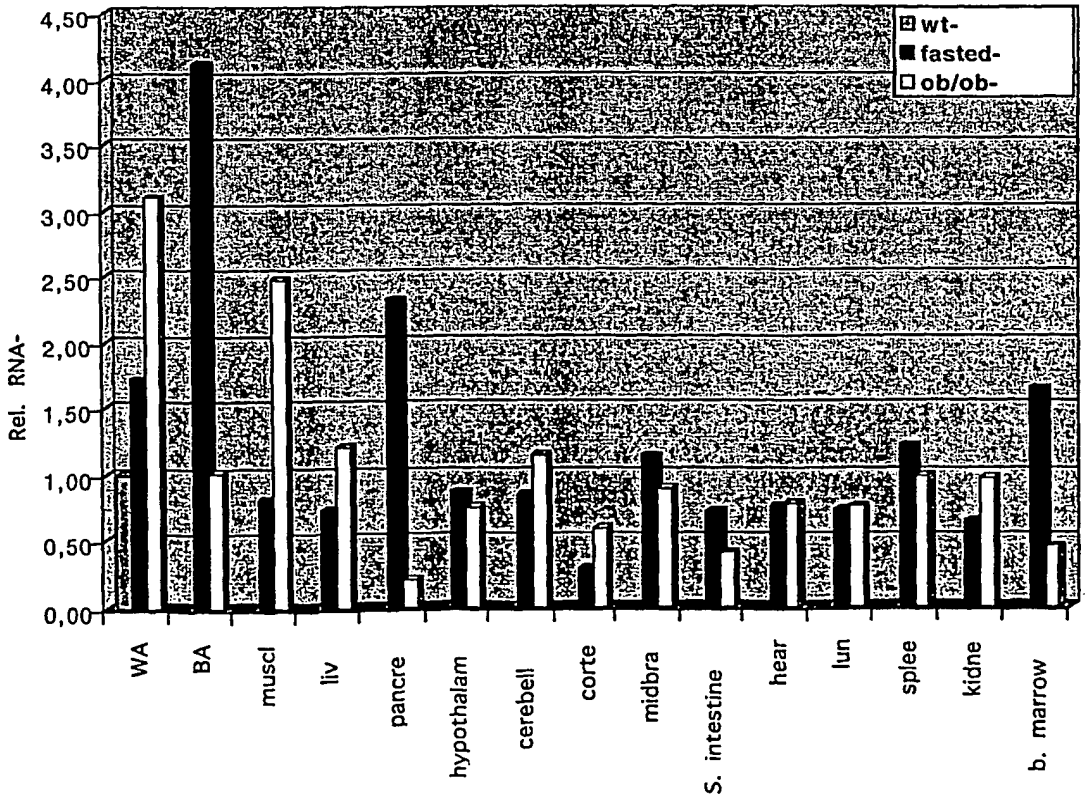
```
hDevG22          ----------MAAFSVGTAMNASSYSAEMTEPK--------------------SVCVSVD
mDevG22          ------MACLMAAFSVGTAMNASSYSAAMTEPK--------------------SVCVSVD
DevG22           MTLLPDIKASDAACCVGGANSDASSSSGVCSLKGHHGDPDSGCLGINCCTTAASSELSID

hDevG22          EVVSSN---MEATETDLLNGHLKKVDNNLTEAQRFSSLPRRAAVNIEFRDLSYSVPEGPW
mDevG22          EVVSSN---VDEVETDLLNGHLKKVDNNFTEAQRFSSLPRRAAVNIEFKDLSYSVPEGPW
DevG22           ETSSTSSRGSCELTSKVTNDLNGFQSPNYHQAVAHANFDHCDPVDIQFADVRYTVKKFSF

hDevG22          WR-KKGYKTLLKGISGKFNSGELVAIMGPSGAGKSTLMNILAG-----------------
mDevG22          WK-KKGYKTLLKGISGKFNSGELVAIMGPSGAGKSTLMNILAG-----------------
DevG22           PERKFVTKEILHGLNGSFRSGELTAIMGPSGAGKSTLLNVMSGFCNGRGGKDDAPGGTRV

hDevG22          -----YRETGMKGAVLING--LPRDLRCFRKVSCYIMQDDMLLPHLTVQEAMMVSAHLKL
mDevG22          -----YRETGMKGAVLING--MPRDLRCFRKVSCYIMQDDMLLPHLTVQEAMMVSAHLKL
DevG22           FIAKRYRSTGVSGDIRVNRKPMAPSSERFRQMLCYIHQDDLLRPQLLVGEIMLLAAHLKL

hDevG22          --QEKDEGRREMVKEILTALGLLSCANTRTGSLSGGQRKRLAIALELVNNPPVMFFDEPT
mDevG22          --QEKDEGRREMVKEILTALGLLPCANTRTGSLSGGQRKRLAIALELVNNPPVMFFDEPT
DevG22           GFKVTKAYKMDLIKHILSLLGLDHRYNVPTGKLSGGQKKRLAIALELISNPPVLYLDEPT

hDevG22          SGLDSASCFQVVSLMKGLAQGGRSIICTIHQPSAKLFELFDQLYVLSQGQCVYRGKVCNL
mDevG22          SGLDSASCFQVVSLMKGLAQGGRSIVCTIHQPSAKLFELFDQLYVLSQGQCVYRGKVSNL
DevG22           TGLDSSSCSSCVALLKKLASQGHTIVCTIHQPSALIFEMFDKLYTVVDGHCMYQGPVREL

hDevG22          VPYLRDLGLNCPTYHNPADFVMEVASGEYGDQNSRLVRAVREGMCDSDHKRDLGGDAEVN
mDevG22          VPYLRDLGLNCPTYHNPADFVMEVASGEYGDQNSRLVRAVREGMCDADYKRDLGGDTDVN
DevG22           VPFLADQQLVCPSYHNPADYLLEVAVGEHQRDLNELIHAANK-----------------

hDevG22          PFLWHRPSEEVKQTKRLKGLRKDSSSMEGCHSFSASCLTQFCILFKRTFLSIMRDSVLTH
mDevG22          PFLWHRPAE------------EDSASMEGCHSFSASCLTQFCILFKRTFLSIMRDSVLTH
DevG22           ------------------------------------------------K-YYEDVDR--

hDevG22          LRITSHIGIGLLIGLLYLGIGNEAKKVLSNSGFLFFSMLFLMFAALMPTVLTFPLEMGVF
mDevG22          LRITSHIGIGLLIGLLYLGIGNEAKKVLSNSGFLFFSMLFLMFAALMPTVLTFPLEMSVF
DevG22           HRYMSSDDMARLVESIKENMGGKAVVKTSEALAAFAAAQFSSFDYVKPSPQELALEEIKA

hDevG22          LREHLNYWYSLKAYYLAKTMADVPFQIMFPVAYCSIVYWMTSQPSDAVRFVLFAALGTMT
mDevG22          LREHLNYWYSLKAYYLAKTMADVPFQIMFPVAYCSIVYWMTSQPSDAVRFVLFAALGTMT
DevG22           LSGGP---ESADPDLLEKNLRPQPQ----PLAKAGELARPPNAIRSASFLMQYVLLMQRI

hDevG22          SLVAQSLGLLIGAASTSLQVATFVGPVTAIPVLLFSGFFVSFDTIPTYLQWMSYISYVRY
mDevG22          SLVAQSLGLLIGAASTSLQVATFVGPVTAIPVLLFSGFFVSFDTIPAYLQWMSYISYVRY
DevG22           LICAKRN---------YFLLLAR-------IFSHIFIGVVFGYLYMNVGNNAQSVLGNYVY

hDevG22          GFEGVILSIYGLDREDLHCDIDETCHFQKSEAILRELDVENAKLYLDFIVLGIFFISLRL
mDevG22          GFEGVILSIYGLDREDLHCDIAETCHFQKSEAILRELDVENAKLYLDFIVLGIFFISLRL
DevG22           LYGSTLLLVY-----------TG--KMAVVLTFPLEIDMLTREHFNRWYKLGPYFLSL--

hDevG22          IAYFVLRYKIRAER-
mDevG22          IAYFVLRYKIRAER-
DevG22           -ISFEIPFQVSTAIE
```

FIGURE 18: Expression of DevG22 in mammalian tissues

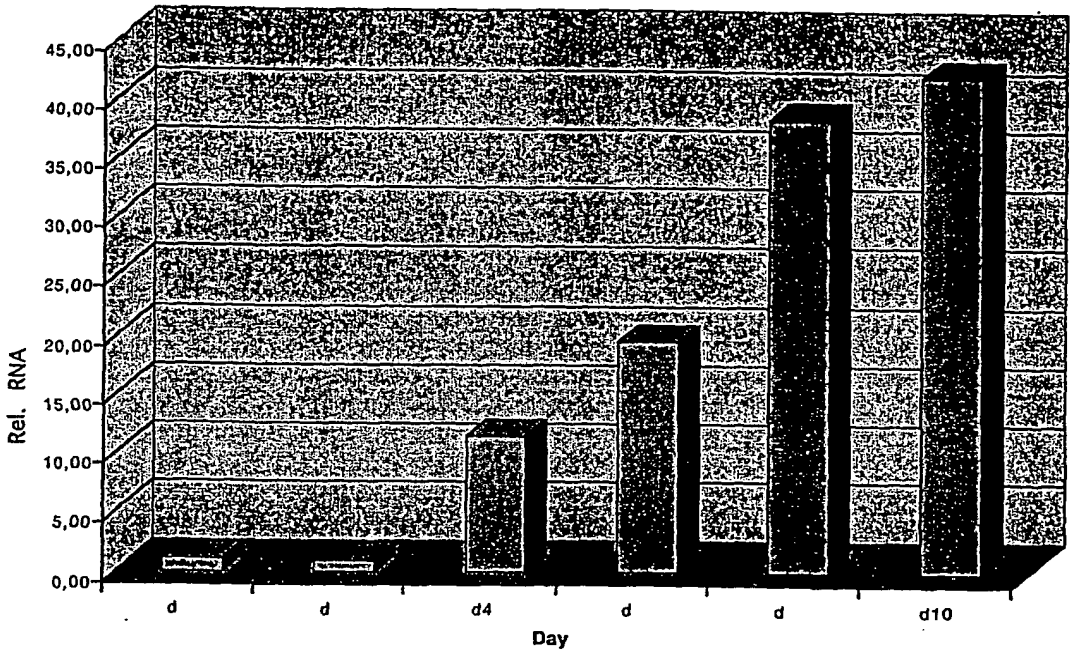
(A) Real-time PCR analysis of DevG22 expression in wildtype mouse tissues



(B) Real-time PCR mediated comparison of DevG22 expression in different mouse models



(C) Real-time PCR mediated comparison of DevG22 expression during the differentiation of 3T3-L1 cells from pre-adipocytes to mature adipocytes.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 October 2002 (10.10.2002)

PCT

(10) International Publication Number
WO 02/079238 A3

(51) International Patent Classification[7]: C12N 9/90, C07K 14/705, A61K 38/52, 38/17, G01N 33/50, C12N 15/62, A01K 67/027, C12N 5/10

(21) International Application Number: PCT/EP02/03540

(22) International Filing Date: 28 March 2002 (28.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01108315.1 2 April 2001 (02.04.2001) EP
01113419.4 1 June 2001 (01.06.2001) EP

(71) Applicant *(for all designated States except US)*: **DEVELOGEN AKTIENGESELLSCHAFT FÜR ENTWICKLUNGSBIOLOGISCHE FORSCHUNG** [DE/DE]; Rudolf-Wissell-Strasse 28, 37079 Göttingen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **EULENBERG, Karsten** [DE/DE]; Am Graben 10b, 37079 Göttingen (DE). **BRÖNNER, Günter** [DE/DE]; Springstrasse 54, 37077 Göttingen (DE). **CIOSSEK, Thomas** [DE/DE]; Kiesseestrasse 49a, 37083 Göttingen (DE). **HÄDER, Thomas** [DE/DE]; Wiesenstr. 17, 37073 Göttingen (DE). **STEUERNAGEL, Arnd** [DE/DE]; Am Kirschberge 4, 37085 Göttingen (DE).

(74) Agents: **WEICKMANN, Franz, Albert** et al.; Weickmann & Weickmann, Postfach 860 820, 81635 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

(88) Date of publication of the international search report:
4 September 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/079238 A3

(54) Title: PROTEIN DISULFIDE ISOMERASE AND ABC TRANSPORTER HOMOLOGOUS PROTEINS INVOLVED IN THE REGULATION OF ENERGY HOMEOSTASIS

(57) Abstract: The present invention discloses three novel proteins regulating the energy homeostasis and the metabolism of triglycerides, and polynucleotides, which identify and encode the proteins disclosed in this invention. The invention also relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides of the invention. The invention also relates to the use of these sequences in the diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation, for example, but not limited to, metabolic diseases such as obesity, as well as related disorders such as adipositas, eating disorders, wasting syndromes (cachexia), pancreatic dysfunctions (such as diabetes mellitus), hypertension, pancreatic dysfunctions, arteriosclerosis, coronary artery disease (CAD), coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, cancer, e.g. cancers of the reproductive organs, sleep apnea, and others.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03540

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/90 C07K14/705 A61K38/52 A61K38/17 G01N33/50
C12N15/62 A01K67/027 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, BIOSIS, MEDLINE, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WETTERAU ET AL.: "Protein disulfide isomerase appears necessary to maintain the catalytically active structure of the microsomal triglyceride transfer protein" BIOCHEMISTRY, vol. 30, 1991, pages 9728-9735, XP002231796 the whole document ---	1-12
A	YOUNG &FIELDING: "The ABCs of cholesterol efflux" NATURE GENETICS, vol. 22, August 1999 (1999-08), pages 316-318, XP002231797 the whole document ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 April 2003	11. 04. 03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Kalsner, I

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03540

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 02 20731 A (BAYER AG ;XIAO YONGHONG (US)) 14 March 2002 (2002-03-14) claims 10-13,36,45,49,68; examples 3-6	1-21,25, 26
X	RITCHIE P J ET AL: "Baculovirus expression and biochemical characterization of the human microsomal triglyceride transfer protein." THE BIOCHEMICAL JOURNAL. ENGLAND 1 MAR 1999, vol. 338 (Pt 2), 1 March 1999 (1999-03-01), pages 305-310, XP002231798 page 308, right-hand column, paragraph 4	1
X	WO 00 58471 A (FRIDLAND ARNOLD ;SCHUETZ JOHN (US); ST JUDE CHILDRENS RES HOSPITAL) 5 October 2000 (2000-10-05) SEQ ID NO: 27 abstract	1-12
X	LEE K ET AL: "ISOLATION OF MOAT-B, A WIDELY EXPRESSED MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN/CANALICULAR MULTISPECIFIC ORGANIC ANION TRANSPORTER-RELATED TRANSPORTER" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 58, no. 13, 1 July 1998 (1998-07-01), pages 2741-2747, XP000876821 the whole document	1-12
X	VENKATESWARAN A ET AL: "Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages: A transcriptional role for specific oxysterols" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 275, no. 19, 12 May 2000 (2000-05-12), pages 14700-14707, XP002214935 the whole document	1-12

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03540

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KLUCKEN J ET AL: "ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 97, no. 2, 18 January 2000 (2000-01-18), pages 817-822, XP002186799 the whole document	1-12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/03540

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.: 22-24
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-21, 25, 26, all partially

   A pharmaceutical composition comprising a nucleic acid molecule of a protein disulfide isomerase (DevG20) of SEQ ID NO:1, the composition for the manufacture of an agent for diagnosis or treatment, the use of the nucleic acid sequence for controlling the function of a gene, which is influenced or modified by DevG20; use for identifying substances which interact with DevG20; a non-human transgenic animal exhibiting amodified expression of DevG20; a method of identifying a polypeptide involved in regulation of energy homeostasis; method of screening for an agent which modulates the interaction of DevG20; a kit comprising a DevG20 nucleic acid molecule.

2. Claims: 1-21, 25, 26, all partially

   as above, but with respect to SEQ ID NO:3 and DevG4

3. Claims: 1-21, 25, 26, all partially

   as above, but with respect to SEQ ID NO:5 and DevG22

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 22-24

Present claims 22-24 relate to a method/use involving a compound merely defined by reference to a desirable characteristic or property, namely its ability to bind to or modulate the activity of DevG20, DevG4 or DevG22.

The claims cover all products/methods having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for such products/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for claims 22-24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/03540

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0220731 A	14-03-2002	AU 8986401 A	22-03-2002
		WO 0220731 A2	14-03-2002
WO 0058471 A	05-10-2000	AU 4052200 A	16-10-2000
		WO 0058471 A2	05-10-2000

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☑ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.